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POTENTIALITIES OF ERADICANT FUNGICIDES FOR COMBATING APPLE SCAB AND SOME OTHER PLANT DISEASES[1]

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In a recent paper, one of the writers (*15*)[3] called attention to the current reliance on protectant spraying as the dominant measure for control of many fruit diseases, and suggested the desirability of increased effort to develop control programs of complementary procedures based on the principles of immunization and eradication, as well as protection. The purpose of the present paper is to report the progress of studies of potentialities for increased application of the principle of eradication to the control of certain types of plant disease through the use of eradicant[4] fungicides. The experimental basis of several brief preliminary reports on this work (*13*, *14*, *15*, *16*, *17*, *18*, *19*, *20*, *25*) is recorded herein.

STUDIES ON APPLE SCAB

INTRODUCTION

Apple (*Malus sylvestris* Mill.) scab, caused by *Venturia inaequalis* (Cke.) Wint., is the most widespread and important disease of deciduous orchard fruits. Despite the excellent progress that has been made in developing measures for its control, it continues to take a heavy toll from the growers and consumers of apples.

Since the advent of bordeaux mixture, protectant spraying (or dusting) has been the dominant measure for apple scab control. So great has been its success that comparatively little attention has been given to the development of complementary measures.

Failures of approved spraying practices to give adequate scab control in Wisconsin led one of the writers and his associates (*10*, *16*, *24*, *33*) to undertake a series of studies relating to the epidemiology and control of this disease. The earlier work (*16*) indicated (1) that, under the conditions encountered in this State, ascospores comprise the only important primary inoculum, and (2) that the quantitative level of the ascosporic inoculum is of prime importance in relation to epidemiology and control. It was further shown that protectant spraying is adapted primarily to control of the disease on the fruit, and ordinarily fails to control leaf infection sufficiently to preclude the development of a dangerous ascosporic inoculum in the following spring. Since all the blossoms expand in a relatively short time, it is usually feasible to keep them and the developing fruits

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[2] Grateful acknowledgments are made to Drs. E. E. Wilson and J. M. Hamilton for collaboration in the earlier work on apple scab, and to other members of the Department of Plant Pathology for assistance in performing the spraying experiments and recording the results.

[3] Reference is made by number (italic) to Literature Cited, p. 435.

[4] It is obvious that some eradicant fungicides may have protectant value, and vice versa. In this paper fungicides are referred to as eradicant or protectant according to their dominant role.

adequately protected by fungicidal treatments. However, new leaves are produced during a much longer period, and unprotected leaf surface may be exposed between fungicidal treatments of any feasible frequency. The current method of scab control by protectant fungicides is, therefore, based chiefly on a philosophy of defense. The pathogen is permitted to maintain a high survival level, and under conditions of moderate or severe occurrence of the disease there is usually little or no cumulative control (*15, 16*).

These considerations suggested a critical reexamination of the current defensive methods of apple scab control and an exploration of possibilities for developing complementary offensive measures designed for direct attack on the pathogen, with the aim of reducing it to a quantitative level at which it might be more surely and economically controlled.

Despite its great accomplishments, the protectant fungicidal method of scab control has some sharp limitations. The most fundamental one, its failure to reduce the pathogen to a sufficiently low survival level to accomplish satisfactory cumulative control, has just been discussed. A further and increasing difficulty in the protectant program is the conflict between the requirements of sufficient toxicity for adequate control of the disease and freedom from objectionable host injury. Increasing age of orchards and concentration of apple culture tend to increase the severity of the disease and, consequently, the need for protection against the fungus. At the same time, rising standards of quality of fruit and increased knowledge of effects of host injury make for lessening the tolerance of toxicity to the host. Considerations of host injury have led to the use of progressively weaker fungicides. Bordeaux mixture has largely been replaced by lime-sulphur, which in turn is being replaced by less toxic materials. However, decreased toxicity of the fungicide necessitates increased frequency and cost of application or increased danger of failure to control the disease. Another limitation of programs based too exclusively on the use of protectant fungicides is the constant danger of serious failure to control the disease in the event of unusually severe epidemics or unavoidable dislocations in the program of applications incident to weather conditions, failure of machinery, or other causes. Furthermore, these programs are objectionably laborious, time consuming, and expensive.

In exploring the possibilities of developing control measures to complement protectant spraying, attention was turned first to consideration of means for a direct attack on the pathogen, with the immediate objective of reducing its survival level sufficiently to facilitate control. It was thought that a sufficiently drastic reduction in the ascosporic inoculum might insure the success of the protectant program, even under the most favorable conditions for scab development, and that lessening the severity of the strain on protectant spraying might permit the use of milder fungicides or fewer applications. The nearest feasible approach to local eradication of the pathogen was regarded as the ultimate objective of this line of work.

There have been many previous attempts to apply eradicant measures to apple scab control; but, whether because the idea is impractical or the methods developed were not sufficiently effective, comparatively little progress has been made.

Disposal of the fallen leaves by burning or burying was empirically recommended long before it was proved that the scab fungus over-

winters in them. Turning the leaves under by clean culture before the ascospores are discharged is often recommended, and is generally regarded as a desirable aid to scab control in situations where such cultivation is a feasible and economic horticultural practice. However, there are a great many situations in which this procedure is not feasible; and, furthermore, exact studies of its effectiveness in relation to the epidemiology and control of scab are lacking. The chief limitation to its success in situations where it can be practiced advantageously appears to be its comparative inefficiency as an eradicant measure.

Possibilities of reducing the survival level of the scab pathogen by spraying have received consideration from time to time; but advances in this direction have been sharply limited both by insufficient understanding of basic phenomena in the epidemiology and control of the disease and lack of suitably adapted materials and methods. Experimentation in this direction has hitherto been dominantly concerned with fungicides that were developed primarily for protectant spraying. For reasons that have been stated, efforts to suppress the pathogen through cumulative effects of protectant spraying have met with comparatively little success. Jehle and Cory (*12*), following a suggestion of W. C. Travers, tested the value of an application of bordeaux, 10–10–50, made just after harvest and before many of the leaves had fallen, thinking that it might check spore production in the fallen leaves in the spring. They concluded that, "When no other material was applied except Bordeaux in the fall, there was apparently a slight gain in the number of fruits free from scab, but this increase was so slight that as an added treatment Bordeaux would not be profitable". Curtis (*4*), tested the effects of spraying the leaves on the ground in the spring with lime-sulphur at the strength used on trees in foliage. Three such applications of spray caused a definite reduction in the incidence of foliage infection, whereas a single treatment did not. She states that, "Application of the spray three or four days before rain does not necessarily prevent the discharge of living ascospores; maximum effectiveness of the spray is secured only when it is applied *immediately after rain*". Early in the development of spraying methods for apple scab control, a dormant application of bordeaux was sometimes recommended as a "clean-up" spray; but the results did not justify general adoption of this practice. In situations where the scab pathogen overwinters in twig lesions or bud scales, dormant applications of lime-sulphur or bordeaux are sometimes employed, though fungicides better adapted to the purpose would seem desirable.

During the course of the work reported in the present paper (cf. *16*, *19*, *20*), Folsom and Ayers (*6*) found that an application of copper sulphate solution, 5–50, after harvest apparently caused no reduction in the development of ascocarps of the scab fungus in the following spring. Wiesmann (*31*) states that, in similar small-scale experiments in one season, each "1% Helion-Winter, Ciba 1930" and "8% Obstbaumkarbolineum Maag" hindered or prevented the formation of perithecia, whereas "20% Schwefelkalkbrühe Siegfried" did not. He reports further that treating the leaves with "5% Obstbaumkarbolineum" as they lay on the ground under the trees in the spring killed 85.6 percent of the ripe perithecia.

In the present investigation eradicant chemical treatments have been directed against the pathogen at two seemingly vulnerable stages

in its life history. The first of these is the period after harvest but before many leaves have fallen. At this time the leaves can still be thoroughly covered by spray and the trees will tolerate a much more drastic treatment than is feasible earlier in the growing season. The other vulnerable stage is after leaf fall but before ascospores are discharged. Still more drastic treatments can then be given to the leaves on the ground. The work on fall spraying is reported first.

THE EARLIER EXPERIMENTS ON FALL SPRAYING, 1924-31

Studies of the possible adaptation of a fall application of eradicant fungicides to apple scab control were started in 1924, and have been continued in each succeeding year. Since the chief purpose of the earlier work (1924–31) was to narrow the problem, it will not be reported in detail. A brief account of it follows.

Branches or whole trees bearing diseased leaves were sprayed after harvest and before many leaves had fallen, and representative samples of sprayed and unsprayed leaves were picked, shortly before they would have fallen, and overwintered on sod in cloth net bags. In the following spring, by means of microscopic examination, records were made on the occurrence of perithecia of *Venturia inaequalis*.

The calcium, copper, and lead arsenites used prior to 1930 and the magnesium arsenite were prepared in the laboratory. The mono- and dicalcium arsenites and the dicalcium arsenate used in 1930, which were not free from admixtures of homologous calcium arsenites or arsenates, respectively, were obtained from manufacturers. The other materials used were obtained from commerical sources.

Experiments of 1924.—A great reduction in the number of perithecia followed the use of each copper sulphate, 1 percent; sodium nitrate, 15 percent; neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) plus sodium aresnite, ¼, ½, and 1 percent, respectively; and certain highly alkaline copper sulphate-potassium hydroxide mixtures. Abundant perithecia developed in the unsprayed leaves and those that had been sprayed with each bordeaux mixture, 6–6–50; lime-sulphur, 1–10; 1 mixture of bordeaux and lime-sulphur, each at the strength just stated; bordeaux, 6–6–50, plus a proprietary sodium polysulphide spray at the strength recommended for dormant spraying; neutral bordeaux (using 1½ percent $CuSO_4.5H_2O$) plus mercuric chloride, 1–1,000; bordeaux 6–6–50, plus certain additions of sodium or potassium hydroxide; neutral bordeaux (using 1½ percent $CuSO_4.5H_2O$) plus a proprietary casein-lime spray supplement; and the last mentioned mixture plus copper sulphate, three-fourths of 1 percent. Severe host injury resulted from many of these sprays, especially copper sulphate and the preparations containing sodium arsenite.

Experiments of 1925.—Comparatively high effectiveness against the fungus was shown by each sodium arsenite, one-eighth of 1 percent; paris green, 1 percent; these two materials at the same rates, used together; bordeaux, 4–4–50, plus paris green, 1 percent; neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) plus calcium arsenite, 1 percent; calcium arsenite, one-half of 1 percent; and calcium arsenite, 1 percent, plus sodium arsenite, one-fourth of 1 percent. In many cases no perithecia developed in the treated leaves. They developed fairly abundantly in the untreated leaves. Most of the treatments were seriously injurious to the host.

Experiments in 1926.—Several mixtures of calcium arsenite, ½ or 1 percent, and neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) or lime, one-half of 1 percent, greatly reduced the number of perithecia without drastic injury to the host. Similar mixtures in which paris green replaced the calcium arsenite gave less control and more host injury. Sodium and calcium silicofluoride, respectively, 1 or 2 percent, occasioned little reduction in the number of perithecia. Perithecia developed abundantly in the untreated leaves.

Experiments in 1927.—Various mixtures of calcium arsenite with bordeaux, or with lime plus tricalcium phosphate (the latter added because of its possible influence on reaction of the spray residues after weathering), were highly effective in preventing the production of perithecia, some of them causing little host injury. Similar mixtures with paris green as the arsenite were less effective. Zinc arsenite was comparatively ineffective, whether used alone or in mixtures with acetic acid or tricalcium phosphate. Pine oil in concentrations up to 10 percent was ineffective. Perithecia developed abundantly in the untreated leaves.

Experiments in 1928.—Calcium, zinc, copper, magnesium, and lead arsenites, respectively, were ineffective in suppressing the perithecia at concentrations tolerated by the host. Bordeaux, 3–2–50, plus calcium arsenite, 1 percent, reduced the number of perithecia by 96 percent. The same reduction was effected by a mixture of calcium arsenite, 1 percent; lime one-fourth of 1 percent; tricalcium phosphate, one-fourth of 1 percent; and a proprietary casein-lime spray supplement, one-sixteenth of 1 percent. Perithecia developed abundantly in the untreated leaves.

Experiments in 1929.—Numerous mixtures of bordeaux, 3–2–50, and calcium arsenite completely suppressed the development of perithecia. When the calcium arsenite component was one-fourth of one-half of 1 percent, the number of perithecia was reduced by from 91 to 99.8 percent, without severe host injury. Certain mixtures of bordeaux and copper arsenite gave very similar results. A casein-lime spray supplement did not significantly improve the effectiveness of the copper-lime-arsenic mixtures, but a summer spray miscible oil showed some promise as a supplement. Perithecia developed abundantly in the untreated leaves.

Experiments in 1930.—A summer spray miscible oil, 1 percent, was used as a supplement to all the materials tested this year. Tri- and dicalcium arsenate, each used alone or mixed with bordeaux, 3–2–50, were comparatively ineffective. Lead, zinc, and copper arsenites and paris green, each used alone in concentrations of ½ or 1 percent, gave unsatisfactory results. If the perithecia were efficiently suppressed, there was too much host injury. In mixtures with bordeaux, 3–2–50, dicalcium arsenite seemed slightly less effective than monocalcium arsenite. Such mixtures as copper sulphate, three-fourths of 1 percent, lime, one-half of 1 percent, and monocalcium arsenite, one-half of 1 percent, gave good suppression of the perithecia, without severe host injury. Similar results were obtained by reducing the monocalcium arsenite to one-fourth of 1 percent, and adding the less soluble zinc or tricalcium arsenite, 1 percent. The development of perithecia in the untreated leaves was less abundant than usual.

Discussion.—The earlier experiments showed that the commonly used protectant fungicides that were tried have little value for the

purpose in view. Various chemical agents known to be toxic to fungi or other plants were then tested. Arsenites were found to be highly effective in suppressing the perithecia; but they were objectionably injurious to the host and had unsatisfactory physical properties when used alone or with the spray supplements tried. Mixtures of certain arsenites and bordeaux were the most promising materials tested. They were found to be highly effective against the fungus, good in physical properties, less injurious to the host than arsenites used alone at effective concentrations, and comparatively cheap. Arsenites of a wide range of solubility are available, and it was early apparent that the toxic properties of copper sulphate-lime-arsenite mixtures could be varied through a wide range by suitable choice of the arsenite and modifications in the concentrations and proportions of the ingredients.

FURTHER EXPERIMENTS ON FALL SPRAYING, 1931-36

The earlier work having demonstrated that a fall application of a suitable spray may be highly effective in preventing the development of perithecia of the scab pathogen, attention was next directed to a more intensive study of the comparative values of some of the more promising materials.

Comparison of Fungicidal Materials

MATERIALS AND METHODS

Arsenical compounds.—All the arsenical compounds used, which were obtained from commercial companies, were finely ground, usually being capable of passage through a 300-mesh sieve. Results of analyses [5] made according to the methods of the Association of Official Agricultural Chemists (*1*) are shown in table 1, with records of the years in which the several compounds were used and the symbols by which they are designated, for brevity, in the spray formulas. The percent of moisture is based on the original air-dry samples; that of each water-soluble and total arsenic, on the oven-dry (110° C.) samples.

TABLE 1.—*Moisture, water-soluble arsenic, and total arsenic content of arsenical compounds used in the experiments of 1931–36*

Material	Years used	Symbol [1]	Moisture	Water soluble arsenic (As_2O_3)	Total arsenic (As_2O_3)
			Percent	*Percent*	*Percent*
Monocalcium arsenite	1931	Cai			
Do	1932	Cai	0.4	24.46	72.86
Do	1933	Cai	.2	21.15	74.97
Do	1934–35	Cai	.9	23.11	74.59
Dicalcium arsenite	1931–34	2 Cai	4.5	14.86	60.61
Tricalcium arsenite	1931	3 Cai			
Do	1932–33	3 Cai	1.0	1.69	55.06
Do	1934	3 Cai	7.0	5.09	31.45
Zinc arsenite	1932	Zai	.1	.29	40.68
Do	1933–35	Zai	.4	.59	41.73
Zinc arsenite, buffered	1935	B Zai	.1	.06	29.75
Copper arsenite	1931	Copai			
Do	1933	Copai	1.3	1.04	38.88
Do	1934	Copai	2.2	2.02	40.59
Do	1935	Copai	4.3	.96	27.96
Paris green	1931–32	P G	.3	.15	57.48
Iron arsenite	1933	Iai	4.2	2.42	39.22
Do	1934	Iai	5.6	2.99	41.60
Lead arsenite	1933–34	Lai	.3	2.93	42.71
Magnesium arsenite	1933–34	Mai	4.2	42.35	74.47
Dicalcium arsenate	1931	2 Caa			
Do	1932	2 Caa			
Do	1933	2 Caa	.6	[2] 12.83	[2] 50.61
Tricalcium arsenate	1931	3 Caa			

[1] For the sake of brevity these symbols are used in the spray formulas. [2] Expressed as As_2O_5.

[5] These determinations were made by C. N. Clayton under the supervision of Prof. V. W. Meloche.

Copper sulphate.—Technical copper sulphate crystals.

Lime.—A lump lime containing not less than 99 percent of calcium oxide.

Fish oil.—Cold-pressed menhaden fish oil.

Casein-lime.—A commercial spray supplement.

Summer spray miscible oil.—A commercial product.

Experimental trees.—For the applications of 1931–33, trees of either Plumb Cider or an unknown variety, about 20 years old, of low to moderate vigor and in sod, were used. For the later work, Dudley trees in a vigorous young orchard, planted in 1931 and cultivated each year, were employed. Summer spraying of these trees was omitted, in order that the leaves might be severely scabbed. The work was centered mainly on the Plumb Cider and Dudley varieties, because the previous experience had shown these to be the most difficult, of the varieties tested, on which to suppress perithecial development. The experimental conditions for suppression of the fungus were, therefore, much more severe than would ordinarily be encountered in commercial orchards.

Mixing the sprays.—The required amount of a 10-percent aqueous solution of copper sulphate was placed in a mixing pail containing about three-fourths of the total volume of water, and milk of lime (containing 10 percent CaO) was added. The arsenical compounds, which had been made into a thin paste with water, were then added, followed by the spray supplement, if any, and the remainder of the water. The mixtures were vigorously agitated immediately after each addition of material, and applied promptly.

Applying the sprays.—The sprays were applied to selected branches by means of 6- to 8-liter hand sprayers. Care was taken to cover all the leaves as thoroughly as feasible on both sides and to avoid drift of sprays to other experimental branches. Nearly all applications were made during the first week of October. The treatments were made on dry foliage and under conditions that permitted the spray to dry before rain occurred, as slow drying tends to increase host injury by copper-lime-arsenic mixtures.

Overwintering the leaves.—In all the experiments reported in tables 2 and 3, the leaves were overwintered in cloth mesh bags (fig. 1, *A*), laid on sod in the orchard and fastened at the corners with large nails driven into the soil. Ordinarily from 30 to 50 leaves that showed abundant scab infection on the dorsal surface were placed in each bag, and in many cases the samples were laid down in duplicate or triplicate. The leaves were not allowed to overlap each other, and all were exposed with the dorsal surface up. In nature, the perithecia tend to be borne more abundantly on the dorsal surface. An examination of overwintered Dudley leaves under natural conditions in the spring showed that about 75 percent lay with the dorsal surface up. The overwintered experimental samples were allowed to remain in the field in the spring as long as was possible without undue deterioration of leaves or ascocarps. They were then stored dry in the laboratory. In the dry springs of 1934 and 1936, in order to give the ascocarps more favorable opportunity for development, all the samples were moistened with distilled water when they were brought from the field, and held in a cool chamber for several days.

In 1934–35, in order to determine whether substantially different results might follow if the treated leaves were overwintered with the

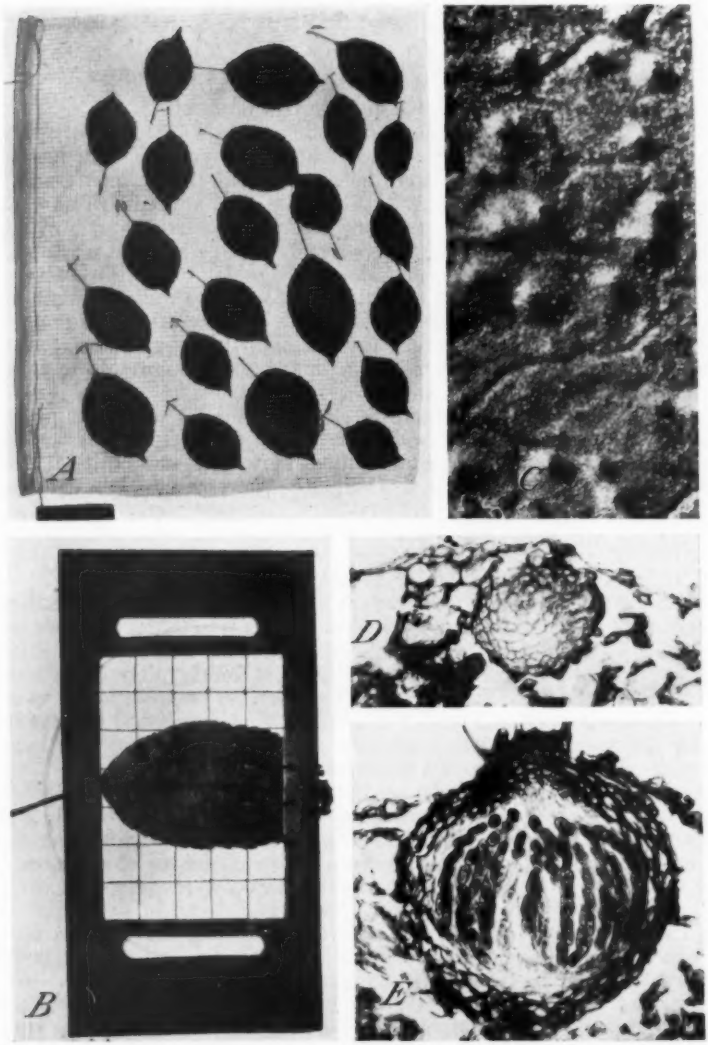

FIGURE 1.—*A*, Apple leaves in open-mesh cloth bag ready for overwintering; *B*, leaf and measuring device in position for counts of perithecia; *C*, apple leaf showing perithecia of *Venturia inaequalis* at the magnification (× 20) at which the counts were made; *D*, section of a treated leaf (formula 139 of table 2) showing a disorganized ascocarp initial, × 425; *E*, section of an untreated leaf (control on *D*) showing a normal perithecium, × 425.

ventral surface up, samples from 26 different treatments were placed on the sod in duplicate with the dorsal surface up in one series and the ventral in the other. The results of the two series were closely similar.

In 1935–36 the development of perithecia in leaves overwintered by the standard method was compared with that in similar leaves overwintered in bags laid on the surface of freshly cultivated soil. In both the treated and untreated samples, somewhat more perithecia were produced in the leaves that lay on the soil than in those on the sod. The spring of 1936 being comparatively dry, the greater moisture accessible to leaves on the soil was beneficial to the fungus. In wet years, however, many leaves thus exposed would disintegrate before the ascospores were discharged.

Counting the perithecia.—Results were usually taken on 20 to 30 leaves per sample. Occasionally disintegration necessitated using a smaller number. After having been soaked in water for a few minutes, the leaf was appressed, weathered surface up, to a small plate of glass and the surplus water absorbed by a wad of cheesecloth. Under the dissecting microscope counts were made of the visible perithecia in ¾-inch areas of leaf surface that bore them in greatest number. Crossed wires held in a rectangular brass frame that was laid on the leaf made it possible to measure the desired areas conveniently without duplication (fig. 1, *B*). Perithecia at the magnification at which the counts were made are shown in figure 1, *C*. Identification of the perithecia was facilitated by pricking out doubtful bodies with a dissecting needle, and examining them under higher magnification if necessary. A disorganized ascocarp initial in a section of a treated leaf is illustrated in figure 1, *D*. Such bodies are not visible from the surface, and are not included in the counts. Figure 1, *E*, illustrates a normal perithecium in a section of an untreated leaf. Most of the samples were examined by more than one worker and without knowledge of the treatment. The results of the counts are expressed as the average number of perithecia per square inch of leaf surface on which the counts were made.

When perithecia occurred in leaves that had received treatments of comparatively high efficiency, they were commonly found in localized areas small enough to be fully included in the counts. The counted perithecia of the controls, however, ordinarily included only a small percentage of the entire number visible on the leaf. Comparisons based on the total number for the entire leaf area, therefore, would indicate a substantially higher degree of effectiveness for the more efficient treatments than is shown by the method used.

ENVIRONMENTAL CONDITIONS

The development of perithecia is greatly influenced by environmental conditions, especially moisture and temperature (*33*). Figure 2 shows daily rainfall and maximum and minimum temperatures recorded by the Madison station of the United States Weather Bureau from September 19 to May 21, in the years 1931–36.

Though temperature is a cardinal factor conditioning perithecial development, it is not ordinarily a sharply limiting one in Wisconsin. Moisture relations, however, are of prime importance in determining the quantity of perithecia produced. Furthermore, they exert an important influence on the effectiveness of the eradicant fungicidal treatments. Enough moisture is essential to dissolve the materials employed sufficiently for them to exercise their toxic effects. However too high rate of loss of the spray residues limits the effectiveness of the treatments.

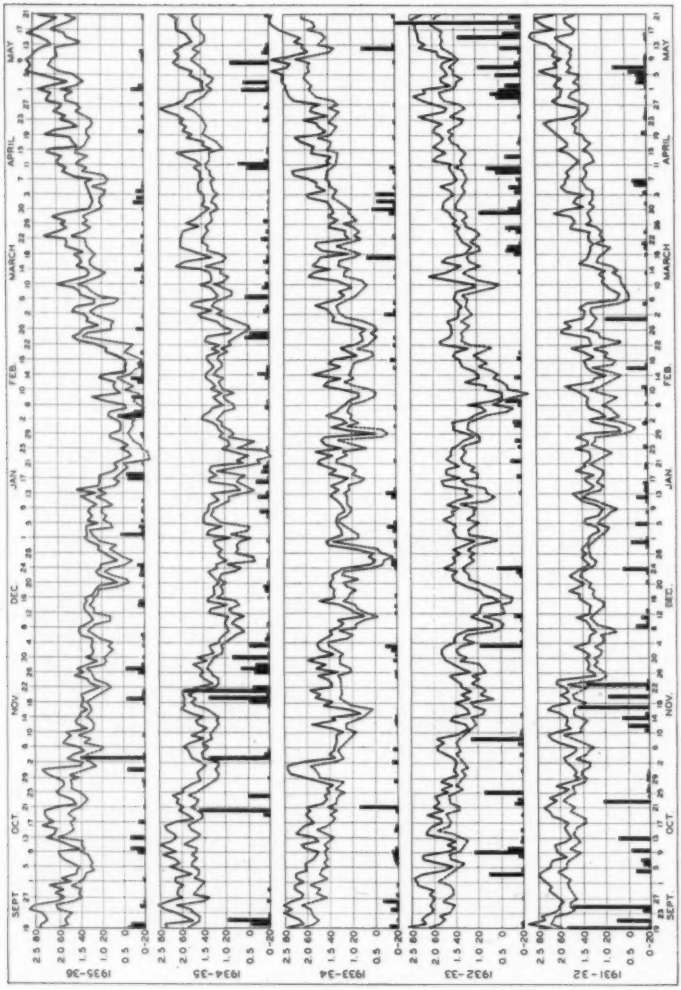

FIGURE 2.—Summary of temperature and rainfall records pertinent to the studies of eradicant spray treatments in relation to apple scab control, Madison, Wis., 1931–36.

In 1931–32, 1933–34, and 1935–36 suppression of the perithecia by eradicant sprays was much easier than in 1932–33 and 1934–35. In 1931–32 the rainfall, though slightly heavier than average, was fairly evenly distributed. In 1932–33 rainfall was slightly above normal in the fall and nearly twice normal in the spring. Rainfall was light in 1933–34, and hot, dry weather in the spring was unfavorable for the fungus. In 1934–35 no rain fell while the treated leaves remained on the trees; but over 10 inches fell during October and November and ample rains occurred in the spring. There were no heavy rains in the fall of 1935, and the following spring was comparatively dry.

EXPERIMENTAL RESULTS

Data on the comparative effectiveness of the experimental materials in suppressing the development of perithecia of *Venturia inaequalis* appear in table 2.

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*

Formula [1]	Incidence of perithecia in percent [2] of the number [3] found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
1. Untreated	100	100	100	100	100	100
2. CS3/4+L1/2+FO1/4	20	16	92	123	55	
3. CS3/4+L1/2+FO1/4					52	
4. CS1+L1+FO1/2			78			
5. CS1+L1/2+FO1/4				106	67	
6. CS1+L1/4+FO1/4				88	49	
7. CS1+L1/2+FO1/4				129	60	
8. CS1+L1/2+FO1/4				123	45	
9. CS1 1/4+L (neut.)+FO1/4			71			
10. CS1 1/2+L1+FO5/8			86			
11. CS1 1/2+L3/8+FO3/8				75	99	
12. CS3/4+L3/4+Cal 1/2+FO1/2			12		5	
13. CS3/4+L1/2+Cal 1/4+FO3/8	2	17	36			
14. CS3/4+L1/2+Cal 3/8+FO3/8	3	21	20		16	
15. CS3/4+L1/2+Cal 1/2+FO3/8		3	19	T		0
16. CS3/4+L1/2+Cal 3/4+FO1/2			20		14	
17. CS3/4+L4/10+Cal 3/8+FO3/8					6	
18. CS3/4+L3/18+Cal 1/4+FO1/4					8	
19. CS3/4+L3/18+Cal 3/8+FO1/4					11	
20. CS3/4+L1/2+Cal 1/8+FO1/4					7	
21. CS3/4+L1/2+Cal 1/4+FO1/4					10	
22. CS3/4+L1/2+Cal 3/8+FO1/4					13	
23. CS1+L1+Cal 1/2+FO5/8			40	5		
24. CS1+L1+Cal 5/8+FO5/8			25			
25. CS1+L1+Cal 3/4+FO5/8			12		3	6
26. CS1+L1+Cal 1+FO3/4			17			
27. CS1+L3/4+Cal 1/2+FO1/2			4			1
28. CS1+L3/4+Cal 3/8+FO1/2			3			
29. CS1+L3/4+Cal 3/4+FO5/8			9		5	
30. CS1+L1/2+Cal 1/4+FO3/8					22	
31. CS1+L1/2+Cal 1/2+FO1/2			4	1	4	
32. CS1+L1/2+Cal 3/4+FO1/2			26			0
33. CS1+L1/2+Cal 1/4+FO1/2				2	30	
34. CS1+L1/2+Cal 3/8+FO1/2				3		
35. CS1+L1/2+Cal 1/2+FO1/2				T		
36. CS1+L1/4+Cal 1/8+FO1/4					24	
37. CS1+L1/4+Cal 1/4+FO3/8				3	26	1
38. CS1+L1/4+Cal 3/8+FO3/8				1	8	1
39. CS1+L1/4+Cal 1/2+FO3/8			1	1	3	0
40. CS1+L1/2+Cal 1/4+FO1/4				4		

[1] CS=copper sulphate; L=lime; FO=fish oil; ZO=zinc oxide. For explanation of other symbols see table 1. The numbers refer to the percent by weight, assuming the mixture to have the same weight as water, with the exception of fish oil, for which they refer to percent by volume. In preparing "neutral" bordeaux, milk of lime was added in successive small amounts until the supernatant liquid, after agitation, no longer gave a positive potassium ferrocyanide test for copper. [2] T=trace.

[3] The average numbers of perithecia per square inch of untreated leaf surface examined were: 1931, unknown variety 553, Plumb Cider 375; 1932, Plumb Cider 575; 1933, Plumb Cider 522; 1934, Dudley 1,188; 1935, Dudley 506. See text for account of methods.

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
41. CS1+L1/6+Cai 1/4+FO1/4				2		
42. CS1+L (neut.) +Cai 1/2+FO3/8			1			
43. CS1 1/4+L3/10+Cai 3/8+FO3/8				T		
44. CS1 1/2+L1+Cai 3/4+FO3/4			23		17	2
45. CS1 1/2+L3/8+Cai 1/4+FO1/2					8	
46. CS1 1/2+L3/8+Cai 3/8+FO1/2				T		
47. CS1 1/2+L1/2+Cai 1/2+FO5/8				1		
48. CS3/4+L1/2+2Cai 1/2+FO3/8			3			
49. CS3/4+L1/2+2Cai 3/4+FO1/2			17			
50. CS3/4+L1/2+2Cai 1+FO1/2	1	3	9		5	
51. CS3/4+L1/2+2Cai 1/4+FO3/4					17	
52. CS3/4+L1/2+2Cai 3/8+FO1/4					15	
53. CS1+L1+2Cai 1/2+FO5/8			1		22	
54. CS1+L1+2Cai 3/4+FO3/4			1			
55. CS1+L1+2Cai 1+FO3/4			2			
56. CS1+L1/4+2Cai 1/4+FO3/8					17	
57. CS1+L1/4+2Cai 3/8+FO3/8				11		
58. CS1+L1/4+2Cai 1/2+FO3/8				1		
59. CS3/4+L1/2+3Cai 1/2+FO3/8			5			
60. CS3/4+L1/2+3Cai 3/4+FO1/2			6		14	
61. CS3/4+L1/2+3Cai 1+FO1/2	4	3	2			
62. CS3/4+L1/2+3Cai 3/8+FO1/4					7	
63. CS3/4+L1/2+3Cai 1/2+FO1/4					13	
64. CS1+L1+3Cai 1/2+FO5/8			6			
65. CS1+L1+3Cai 3/4+FO3/4			4	1		
66. CS1+L1+3Cai 1+FO3/4			4	6	12	
67. CS1+L1/2+3Cai 1+FO5/8				0	4	
68. CS1+L1/2+3Cai 5/8+FO1/2				1		
69. CS1+L1/2+3Cai 5/8+FO3/8				4		
70. CS1+L1/4+3Cai 3/8+FO3/8					24	
71. CS1+L1/4+3Cai 1/2+FO3/8				1	24	
72. CS1+L1/4+3Cai 5/8+FO3/8				1		
73. CS1+L1/4+3Cai 3/4+FO1/2				9		
74. CS1+L1/4+3Cai 1+FO1/2				T		
75. CS1 1/2+L3/8+3Cai 1/2+FO1/2						
76. CS1 1/2+L3/8+3Cai 3/4+FO5/8				4		
77. CS3/4+L1/2+Zai 3/4+FO1/2			6			
78. CS3/4+L1/2+Zai 1+FO5/8			18			
79. CS1+L1+Zai 3/4+FO5/8					28	5
80. CS1+L1+Zai 1+FO3/4			2	T	21	0
81. CS1+L1/2+Zai 1+FO3/8				31		
82. CS1+L1/4+Zai 1/2+FO3/8					27	
83. CS1+L1/4+Zai 3/4+FO1/2				T	10	
84. CS1+L1/4+Zai 1+FO1/2				3		
85. CS1+L1/2+Zai 1+FO1/2				2		
86. CS1+L1/6+Zai 1/2+FO3/8				1		
87. CS1+L1/6+Zai 1+FO1/2				1		
88. CS1 1/4+L (neut.)+Zai 1+FO1/2			3			
89. CS1 1/2+L1/2+Zai 3/4+FO3/8					20	
90. CS1 1/2+L1/2+Zai 1+FO3/4				2	16	
91. CS3/4+L1/2+Copai 1/2+FO3/8	7	12				
92. CS3/4+L1/2+Copai 1/2+FO1/4					44	
93. CS3/4+L1/2+Copai 1+FO3/8					10	
94. CS1+L1/4+Copai 1/2+FO3/8					34	
95. CS1+L1/4+Copai 3/4+FO1/2					19	
96. CS1+L1/4+Copai 1+FO1/2				3	23	1
97. CS1+L1/2+Copai 1/2+FO3/8					42	
98. CS1+L1/2+Copai 3/4+FO3/8					42	
99. CS1+L1/2+Copai 1+FO1/2				0	20	
100. CS1+L1/6+Copai 1/2+FO3/8					42	
101. CS1+L1/6+Copai 1+FO1/2				0	13	2
102. CS1 1/2+L3/8+Copai 1+FO5/8				6	20	
103. CS3/4+L1/2+Iai 1/2+FO3/4					34	
104. CS3/4+L1/2+Iai 3/4+FO3/8					40	
105. CS3/4+L1/2+Iai 1+FO5/8					37	
106. CS1+L1/4+Iai 1/2+FO1/2					54	
107. CS1+L1/4+Iai 3/4+FO1/2					26	
108. CS1+L1/4+Iai 1+FO1/2				1	49	
109. CS1+L1/2+Iai 3/4+FO 3/8				1	27	
110. CS1+L1/2+Iai 1+FO1/2				1	31	

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
111. CS1+L1/16+Iai 1/2+FO3/8						
112. CS1+L1/16+Iai 1+FO1/2				42	42	
113. CS1 1/2+L3/8+Iai 1+FO5/8				12	32	
114. CS3/4+L1/2+PG 1/2+FO3/8			67			
115. CS3/4+L1/2+PG 3/4+FO1/2			41			
116. CS3/4+L1/2+PG 1+FO1/2	T	2	30			
117. CS3/4+L (neut.)+PG 1/2+FO1/4			86			
118. CS1+L1+PG 1+FO3/4			13			
119. CS1 1/2+L1+PG 1/2+FO3/4			63			
120. CS1+L1/4+Lai 1+FO1/2				70		
121. CS1+L1/2+Lai 1+FO1/2				13	32	
122. CS1+L1/16+Lai 1+FO1/2				36		
123. CS1 1/2+L3/16+Lai 1+FO5/8					22	
124. CS1 1/2+L3/8+Lai 1+FO5/8				9		
125. CS1+L1/4+Mai 1/4+FO3/8				6	11	
126. CS1+L1/4+Mai 3/8+FO3/8				13		
127. CS1+L1/4+Mai 1/2+FO 3/8				3		
128. CS1+L1/2+Mai 3/8+FO3/8				1	8	
129. CS3/4+L1/2+2 Caa 1+FO5/8	0	4	19			
130. CS3/4+L1/2+2 Caa 1 1/2+FO5/8			9			
131. CS1+L1+2 Caa 1+FO3/4			40			
132. CS1+L1/2+2 Caa 1+FO5/8			8			
133. CS1+L1/4+2 Caa 1+FO1/2			13			
134. CS1+L (neut.)+2 Caa 1+FO1/2			22			
135. CS1 1/2+L1+2 Caa 1+FO7/8			34			
136. CS3/4+L1/2+3Caa 1+FO1/2		34				
137. CS3/4+L1/4+Cai 1/4+Zai 1/2+FO1/2					5	0
138. CS3/4+L1/2+Cai 1/4+Zai 3/4+FO1/2					12	1
139. CS3/4+L1/2+Cai 1/4+Zai 1+FO5/8			T	T		0
140. CS3/4+L1/2+Cai 1/4+Zai 1/2+FO3/8					11	
141. CS1+L1+Cai 1/4+Zai 1/2+FO5/8					13	
142. CS1+L1+Cai 1/4+Zai 3/4+FO3/4					17	0
143. CS1+L1+Cai 1/4+Zai 1+FO3/4				T	6	T
144. CS1+L1+Cai 1/2+Zai 1/2+FO3/4			1			0
145. CS1+L3/4+Cai 1/4+Zai 1/2+FO5/8						0
146. CS1+L3/4+Cai 1/4+Zai 3/4+FO5/8						5
147. CS1+L3/4+Cai 1/4+Zai 1+FO3/4						1
148. CS1+L3/4+Cai 1/2+Zai 1/2+FO3/4			2			
149. CS1+L3/4+Cai 1/2+Zai 1+FO3/4			4			
150. CS1+L1/2+Cai 1/4+Zai 1/4+FO3/8					16	
151. CS1+L1/2+Cai 1/4+Zai 1/2+FO1/2						6
152. CS1+L1/2+Cai 1/4+Zai 3/4+FO5/8						0
153. CS1+L1/2+Cai 1/4+Zai 1 +FO5/8				0		1
154. CS1+L1/2+Cai 1/8+Zai 1/2+FO1/2					9	
155. CS1+L1/2+Cai 1/8+Zai 3/4+FO1/2					9	
156. CS1+L1/2+Cai 1/4+Zai 1/2+FO1/2					18	
157. CS1+L1/2+Cai 1/4+Zai 3/4+FO1/2				9		
158. CS1+L1/2+Cai 1/4+Zai 1+FO5/8				0		
159. CS1+L1/2+Cai 3/8+Zai 3/4+FO1/2				T		
160. CS1+L1/2+Cai 1/2+Zai 1/2+FO1/2				T		
161. CS1+L1/2+Cai 1/2+Zai 3/4+FO1/2				0		
162. CS1+L3/16+Cai 1/4+Zai 1+FO5/8				0		
163. CS1+L1/4+Cai 1/8+Zai 1/4+FO3/8					33	
164. CS1+L1/4+Cai 1/8+Zai 1/2+FO3/8					20	
165. CS1+L1/4+Cai 1/8+Zai 3/4+FO1/2					18	
166. CS1+L1/4+Cai 1/8+Zai 1+FO1/2				T		
167. CS1+L1/4+Cai 1/4+Zai 1/4+FO3/8					22	
168. CS1+L1/4+Cai 1/4+Zai 1/2+FO1/2				1	8	2
169. CS1+L1/4+Cai 1/4+Zai 3/4+FO1/2				2	6	0
170. CS1+L1/4+Cai 1/4+Zai 1+FO5/8				0		0
171. CS1+L1/4+Cai 3/8+Zai 1/2+FO1/2				5		
172. CS1+L1/4+Cai 3/8+Zai 3/4+FO1/2				0		
173. CS1+L1/4+Cai 1/2+Zai 1/2+FO1/2				1		
174. CS1+L1/2+Cai 1/4+Zai 1/2+FO1/2				T		
175. CS1+L1/2+Cai 1/4+Zai 1+FO1/2				0		
176. CS1+L1/16+Cai 1/4+Zai 1/2+FO3/8				0		
177. CS1+L1/16+Cai 1/4+Zai 1+FO1/2				0		
178. CS1 1/2+L1+Cai 1/4+Zai 1/2+FO3/4						1

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
179. CS1½+L1+Cai ¼+Zai ¾+FO⅞						0
180. CS1½+L1+Cai ¼+Zai 1+FO⅞						0
181. CS1½+L⅜+Cai ⅛+Zai ½+FO⅝					29	
182. CS1½+L⅜+Cai ¼+Zai ½+FO⅝					9	
183. CS1½+L⅜+Cai ¼+Zai ¾+FO⅝				T		
184. CS1½+L⅜+Cai ⅜+Zai ½+FO⅝				0		
185. CS1+L1+Cai ½+ZO½+FO¾						T
186. CS¾+L½+Cai ¼+Copai ½+FO½					12	
187. CS1+L1+Cai ½+Copai ½+FO¾						1
188. CS1+L½+Cai ¼+Copai ½+FO½					10	
189. CS1+L½+Cai ¾+Copai ½+FO½					17	
190. CS1+L¼+Cai ⅛+Copai ¼+FO⅜					24	
191. CS1+L¼+Cai ⅛+Copai ½+FO⅜					14	
192. CS1+L¼+Cai ⅛+Copai ¾+FO½					11	
193. CS1+L¼+Cai ⅛+Copai 1+FO½					9	
194. CS1+L¼+Cai ¼+Copai ¼+FO⅜					13	
195. CS1+L¼+Cai ¼+Copai ½+FO½				0	12	
196. CS1+L¼+Cai ¼+Copai ¾+FO½					2	5
197. CS1+L¼+Cai ¼+Copai 1+FO⅝				0		3
198. CS1+L½+Cai ¼+Copai ¾+FO½						1
199. CS1+L½+Cai ¼+Copai 1+FO½						1
200. CS1+L⅝+Cai ¼+Copai ¾+FO½						T
201. CS1+L⅝+Cai ¼+Copai 1+FO½						2
202. CS¾+L½+Cai ¼+Iai ¾+FO½					7	
203. CS1+L½+Cai ¼+Iai ¾+FO½					8	
204. CS1+L¼+Cai ⅛+Iai ¼+FO⅜					16	
205. CS1+L¼+Cai ⅛+Iai ½+FO⅜					29	
206. CS1+L¼+Cai ⅛+Iai ¾+FO½					19	
207. CS1+L¼+Cai ⅛+Iai 1+FO½					31	
208. CS1+L¼+Cai ¼+Iai ¼+FO⅜					20	
209. CS1+L¼+Cai ¼+Iai ½+FO½				T	6	
210. CS1+L¼+Cai ¼+Iai ¾+FO½					15	
211. CS1+L¼+Cai ¼+Iai 1+FO⅝				1	10	
212. CS1½+L⅜+Cai ¼+Iai ½+FO⅝					10	
213. CS1+L¼+Cai ¼+Lai ½+FO⅜					10	
214. CS1+L¼+Cai ⅛+Lai ¾+FO½					10	
215. CS1+L¼+Cai ¼+Lai ¼+FO⅜					22	
216. CS1+L¼+Cai ¼+Lai ½+FO½				0	12	
217. CS1+L¼+Cai ¼+Lai 1+FO½				1		
218. CS¾+L½+Cai ¼+2Cai 1+FO⅝	2	2				
219. CS¾+L½+Cai ¼+3Cai 1+FO⅝	T					
220. CS¾+L½+Cai ¼+2 Caa 1+FO⅝	T					
221. CS1+L1+Cai ¼+2Caa 1+FO¾			16			
222. CS1+L1+Cai ½+2Caa ½+FO¾			13			
223. CS¾+L½+Cai ¼+3Caa 1+FO⅝	3					
224. CS¾+L½+2 Caa 1+PG ¼+FO⅝			18			
225. CS1+L½+2Caa 1+Zai ½+FO¾			4			
226. CS1+L½+2Caa ½+Zai 1+FO¾			2			
227. CS1+L½+2Caa ¾+Zai ¾+FO¾			1			
228. CS1+L¼+Copai ¼+Iai ½+FO½						
229. CS1+L¼+Copai ½+Iai ½+FO½					29	
230. CS¾+L½+Cai ¼+Zai ½+Copai ½+FO⅝					16	
231. CS1+L1+Cai ¼+Zai ⅜+Copai ⅜+FO¾						T
232. CS1+L1+Cai ¼+Zai ½+Copai ½+FO¾						T
233. CS1+L¾+Cai ¼+Zai ⅜+Copai ⅜+FO⅝						T
234. CS1+L¾+Cai ¼+Zai ½+Copai ½+FO¾						T
235. CS1+L½+Cai ¼+Zai ⅜+Copai ⅜+FO⅝						T
236. CS1+L½+Cai ¼+Zai ½+Copai ½+FO⅝						2
237. CS1+L¼+Cai ¼+Zai ⅜+Copai ⅜+FO½						9
238. CS1+L¼+Cai ¼+Zai ½+Copai ½+FO⅝						3
239. CS1½+L1+Cai ¼+Zai ½+Copai ½+FO⅞						0
240. CS1+L½+Cai ¼+ZO½+Copai ½+FO⅝						T
241. CS1+L¼+2Cai ⅜+Zai ½+FO½				0		4
242. CS1+L¼+3Cai ¼+Zai ¾+FO½				4		
243. CS1+L¼+3Cai ¼+Zai 1+FO⅝				2		
244. CS1+L¼+3Cai ⅜+Zai ½+FO½				10		
245. CS1+L¼+3Cai ½+Zai ½+FO½				4		
246. CS1+L¼+3Cai ½+Zai 1+FO⅝				10		
247. CS1½+L⅜+3Cai ⅜+Zai ½+FO⅜				11		

Copper-lime mixtures.—The 10 copper-lime formulas used in the copper-lime-arsenic mixtures were tested without the arsenical components. The results were very irregular, varying greatly from year to year. Occasionally a substantial reduction of perithecia was effected. Usually, however, it was less than 50 percent, and in some instances the treatments appeared to be wholly ineffective. None of these preparations was sufficiently effective to have promise for the purposes of this investigation, except for use in mixtures containing some other toxic component.

Copper-lime-monocalcium arsenite mixtures.—Many of the copper-lime-monocalcium arsenite formulas were highly effective in suppressing perithecial development, but host injury was a limiting factor. Many modifications in the concentration and proportions of the ingredients were tried in the hope of finding a formula that would be effective enough and not too injurious. Lowering the amount of lime when the amounts of copper sulphate and the arsenite remained unchanged increased fungicidal effectiveness and host injury. Formulas 15 and 27 are among the best of this group. The development of more promising formulas has recently led to lessening the work on copper-lime-monocalcium arsenite mixtures.

Copper-lime-di- or tricalcium arsenite mixtures.—These mixtures gave results very similar to those of the copper-lime-monocalcium arsenite preparations. The di- and tricalcium arsenites did not appear to be better adapted than monocalcium arsenite for such mixtures.

Copper-lime-zinc arsenite mixtures.—Some copper-lime-zinc arsenite mixtures showed considerable promise. The zinc arsenite, however, had to be used at a concentration of about 1 percent in order to attain a promising degree of effectiveness, and even at this strength these mixtures did not always satisfactorily suppress the development of perithecia. The preparations of this group were distinctly less injurious to the host than the corresponding mixtures in which the calcium arsenites were used. Formula 80 gave good results in 3 of the 4 years in which it was tried. Less attention has recently been given to formulas of this group, since others have seemed to offer more promise.

Copper-lime-copper arsenite mixtures.—The results from copper-lime-copper arsenite mixtures seemed very promising in 1933–34, but were disappointing in the following year. Preparations containing less than 1 percent of copper arsenite seemed to be of comparatively little value. The low-lime mixtures were more efficient than like formulas with higher lime components. Formulas 96, 99, and 101 are among the more promising of this group. These preparations were less injurious to the host than the corresponding mixtures in which calcium or zinc arsenites were used.

Copper-lime-iron arsenite mixtures.—The results from the copper-lime-iron arsenite mixtures were very similar to those from the corresponding preparations in which copper arsenite was used, but in general showed somewhat less effectiveness against the fungus.

Copper-lime-paris-green mixtures.—The copper-lime-paris-green mixtures seemed to be the least promising of the copper-lime-arsenite preparations thus far discussed.

Copper-lime-lead arsenite mixtures.—Copper-lime-lead arsenite mixtures were not sufficiently effective to be of promise.

Copper-lime-magnesium arsenite mixtures.—The results from copper-lime-magnesium arsenite mixtures were similar to those from corresponding preparations in which monocalcium arsenite was used. There was no evidence that magnesium arsenite is better adapted for the purposes of the experiments than the calcium arsenite.

Copper-lime-di- or tri-calcium arsenate mixtures.—The copper-lime-dicalcium arsenate mixtures substantially reduced perithecial development with comparatively little host injury, but they did not appear to be sufficiently effective for the purpose. Substitution of the tri- for the di-calcium arsenate substantially lessened effectiveness against the fungus.

Copper-lime-monocalcium and zinc arsenite mixtures.—This group, in which both a highly soluble and a slightly soluble arsenite are used, contains some of the most promising preparations found in this study. Formula 139 gave almost perfect suppression of the perithecia in each of the 3 years it was used, but host injury occurred to an objectionable degree. Formula 144 gave good control of the fungus in the 2 years it was tried, with less host injury. Formulas 153, 168, 169, and 170 were all highly effective against the fungus, but caused some injury. In a single year's trial formulas 179 and 180 were highly effective and caused only very slight host injury. Further experiments are necessary before conclusions can be reached as to the relative merits of formulas in this group and whether such mixtures can be recommended for use in orchard practice.

Copper-lime-monocalcium arsenite and zinc oxide mixture.—In a single trial, formula 185 suppressed the fungus satisfactorily without objectionable host injury. Further experimentation will be necessary for evaluating this type of preparation.

Copper-lime-monocalcium and copper arsenite mixtures.—Preparations in this group gave results similar to those from the corresponding mixtures in which zinc arsenite was used (formulas 187, 196, and 197), but the suppression of perithecia tended to be less and host injury was about the same.

Copper-lime-monocalcium and other arsenite mixtures.—Iron or lead arsenite seemed less promising than zinc or copper arsenite for addition to copper-lime-monocalcium arsenite mixtures.

Certain miscellaneous mixtures.—While certain miscellaneous mixtures (formulas 219, 220, 226, and 227) caused a substantial reduction in development of perithecia, they seem to be less promising than many other formulas.

Copper-lime-monocalcium, zinc, and copper arsenite mixtures.—In 1 year's tests, certain of these preparations containing three arsenites were highly effective in suppressing the perithecia, and formulas 232, 233, and 234 caused comparatively little injury. However, it seems unlikely that mixtures in this group have as much promise as the copper-lime-monocalcium and zinc arsenite preparations (e. g., formulas 143 and 144).

Copper-lime-di- or tri-calcium and zinc arsenite mixtures.—These preparations, which were tried only in 1933, a year in which suppression of the perithecia was comparatively easy, seem less promising than corresponding mixtures in which monocalcium arsenite was used.

Discussion.—The comparative effectiveness of the several types of formula used has been briefly discussed in the foregoing paragraphs. The results presented show that it is possible, even under very severe conditions of scab development, to reduce the ascigerous stage of the pathogen to a very low level in leaves that are thoroughly sprayed in the fall with suitable eradicant fungicides, ascospore production having been completely suppressed in many individual experiments. These small-scale experiments, however, having been designed chiefly for surveying the comparative effectiveness of many formulas for suppressing the fungus, leave for further investigation numerous questions relating to the possible adaptation of fall spraying as a practical measure for apple-scab control. Some of these are considered below.

Variations in Time of Application of Spray

In 1933–34, 1934–35, and 1935–36 a series of experiments was performed to gain data on the relation of time of application to the effectiveness of the sprays against the fungus and their injuriousness to the host. Beginning in late September, applications were made on young Dudley and Northwestern Greening trees (set in the orchard in 1931) at intervals of about 1 week by means of a wheelbarrow sprayer. One or two trees of each variety were given each individual treatment, none being sprayed more than once. Samples of leaves were picked and placed for overwintering on the following dates: 1933, from first and second applications, October 19; from third application, October 24; 1934, from first and second, October 17; third, October 22; 1935, from all applications, leaves picked October 19 and laid on sod October 25. The usual methods of overwintering the leaves and taking data were followed. The results relating to suppression of perithecia appear in table 3.

The variations in time of application employed in these experiments showed no striking or consistent influence on either the effectiveness of the sprays in suppressing the development of perithecia or their injuriousness to the host. In 1933–34 the perithecia were so effectively suppressed by all applications that no substantial differences in effectiveness in relation to the time of application could be observed. There was little injury from any of the treatments. In 1934–35 suppression of the perithecia was unsatisfactory. With the aim of lessening the danger of host injury, the formulas were weakened in a season that proved to be unusually favorable for fungus development. Under the conditions encountered, the latest date of application was the most effective and the intermediate date the least effective. It seems more probable that these differences are due chiefly to relations of time of application to rain periods than that they are attributable to the stage of development of host or parasite. There was little injury from any of the treatments. In 1935–36 the latest application appeared to be slightly more effective than the others. Winter injury in this season was so severe that it interfered with interpretation of results of spray injury. No consistent relation between time of application and host injury was noted.

While these data are too limited to be conclusive, they suggest the desirability of delaying the applications as long as feasible without too much leaf fall.

TABLE 3.—*Effects of variations in the time of application of fall sprays on their efficiency in suppressing perithecia of Venturia inaequalis, Madison Wis., 1933–36*

Year and formula [1]	Incidence of perithecia in leaves sprayed at stated dates in percent [2] of the number [3] found in the untreated leaves					
	Dudley			Northwestern Greening		
	Sept. 21	Sept. 29	Oct. 13	Sept. 21	Sept. 29	Oct. 13
1933-34						
1. Untreated	100	100	100	100	100	100
37. CS1+L¼+Cal¼+FO⅜	1	0	0	1	0	10
38. CS1+L¼+Cal⅜+FO⅜	1	0	1	0	2	3
46. CS1½+L⅜+Cal⅜+FO½	1	1	0	0	0	1
168. CS1+L¼+Cal¼+Zal½+FO½	0	0	0	0	1	1
169. CS1+L¼+Cal¼+Zal¾+FO½	0	0	0	0	0	2
170. CS1+L¼+Cal¼+Zal1+FO⅝	0	0	0	0	0	0
172. CS1+L½+Cal⅜+Zal¾+FO½	3	0	0	0	0	0
183. CS1½+L⅜+Cal¼+Zal¾+FO⅝	2	0	0	0	0	0
	Sept. 24	Oct. 8	Oct. 22	Sept. 24	Oct. 8	Oct. 22
1934-35						
1. Untreated	100	100	100	100	100	100
95. CS1+L¼+Copal¾+FO½	11	23	13	19	99	17
107. CS1+L¼+Ial¾+FO½	16	45	3	11	47	12
15. CS¾+L½+Cal½+FO⅜	11	28	7	10	93	15
191. CS1+L¼+Cal⅜+Copal½+FO⅜	24	51	2	30	43	4
205. CS1+L¼+Cal⅜+Ial½+FO⅜	15	27	12	6	58	17
164. CS1+L¼+Cal⅜+Zal½+FO⅜	12	42	11	30	39	22
	Sept. 23	Oct. 5	Oct. 10	Sept. 23	Oct. 5	Oct. 10
1935-36						
1. Untreated	100	100	100	100	100	100
25. CS1+L1+Cal¾+FO⅝	12	14	0	1	1	1
143. CS1+L1+Cal¼+Zal1+FO¾	2	2	1	1	2	1
236. CS1+L½+Cal¼+Zal½+Copal½+FO⅝	0	3	0	1	0	T
197. CS1+L¼+Cal¼+Copal1+FO⅝	4	5	T	25	T	2

[1] For explanation of symbols used see footnote 1, table 2.

[2] T = Trace.

[3] The average numbers of perithecia per square inch of untreated leaf surface examined were: 1933, Dudley 1003, Northwestern Greening 370; 1934, Dudley 493, Northwestern Greening 214; 1935, Dudley 506, Northwestern Greening 364.

SPRAY INJURY TESTS UNDER ORCHARD CONDITIONS

Extensive data on spray injury were taken in connection with the small-scale experiments on suppression of perithecia. However, the conditions of most of these experiments were so unsatisfactory for injury studies that the results relating thereto will not be given in detail. While more extensive studies on injury must await further narrowing of the problem, some experiments have been initiated, as reported below.

Thirteen different copper-lime-arsenic formulas (nos. 15, 24, 39, 80, 95, 107, 143, 164, 169, 170, 191, 197, and 205 of table 2) were applied during the first half of October by power sprayers to orchard trees of eight apple varieties (Wealthy, McIntosh, McMahon, Northwestern Greening, Fameuse, Lowland Raspberry, Dudley, and Yellow Transparent) in 40 treatments at Sturgeon Bay, Gays Mills, or Madison during the period from 1932–33 to 1935–36, inclusive. One or several trees were used per treatment. Examination was made for leaf injury

about 1 or 2 weeks after the sprays were applied and for bud injury the following spring. No drastic leaf injury was observed, except in one case when showery weather prevented prompt drying of the sprays. Observation of the sprayed and unsprayed trees in the spring revealed no evidence of significant spray injury to the buds. Counts relating to bud injury were made only on Wealthy at Madison. These revealed no significant difference between the sprayed and unsprayed trees, except possibly for formula 39 in 1935–36. In this case the untreated trees showed 2 percent and the sprayed 10 percent of dead terminal buds. Winter injury affected both sprayed and unsprayed trees this year.

These results, though encouraging, are not interpreted as establishing the safety of these formulas for general use as fall sprays for apples.

Effects of Eradicant Spraying on the Epidemiology of Scab

The work thus far reported seems to indicate substantial possibilities for the development of a fall spray treatment capable of reducing the ascosporic inoculum to the neighborhood of 1 percent, or less, of that from similar untreated leaves. The effects of such reductions on the epidemiology and control of the disease remain to be considered. Only a small beginning has been made in experimentation in this important field. Studies of the effects of certain fall spray treatments on the development of the disease in the following year were begun at Madison in 1934. A brief report of the progress of this work follows:

Orchard no. 1 of figure 3, set in 1930, received the fall sprays. Orchard no. 2, set in 1931, was untreated. Both orchards were vigorous. Neither received summer sprays. Cultivation of both was postponed until ascospore discharge was over.

Experiment of 1934–35

On October 9, 1934, all of orchard no. 1 was sprayed by means of a power machine, formulas 191, 164, and 205 of table 2 being used on different subplots.

The environment in fall, winter, and spring was favorable for overwintering of the fungus. Examinations in the spring showed that the sprayed orchard was very nearly free from perithecia of the scab fungus, whereas a fair amount of ascosporic inoculum had developed in the unsprayed. Ascospores were mature by the time the leaf buds opened, and several days of rain and low temperature afforded favorable conditions for early infection (fig. 4). Scab lesions were first observed on May 29, when a wave of disease began to appear. Rains in June and July permitted further infection periods, and the season was generally favorable for scab development.

Comparative data on scab development in the two orchards appear in tables 4 and 5. The results shown in table 4 indicate that through the month of June reduction of scab infection in the sprayed orchard was not less than 90 percent. The sprayed Wealthy block showed 92 percent scab-free fruit on June 24, as compared with 48 percent from the unsprayed (table 5). At harvest 89 percent of the fruit from the sprayed block was free of scab, while the unsprayed block had insufficient fruit for counts at that time.

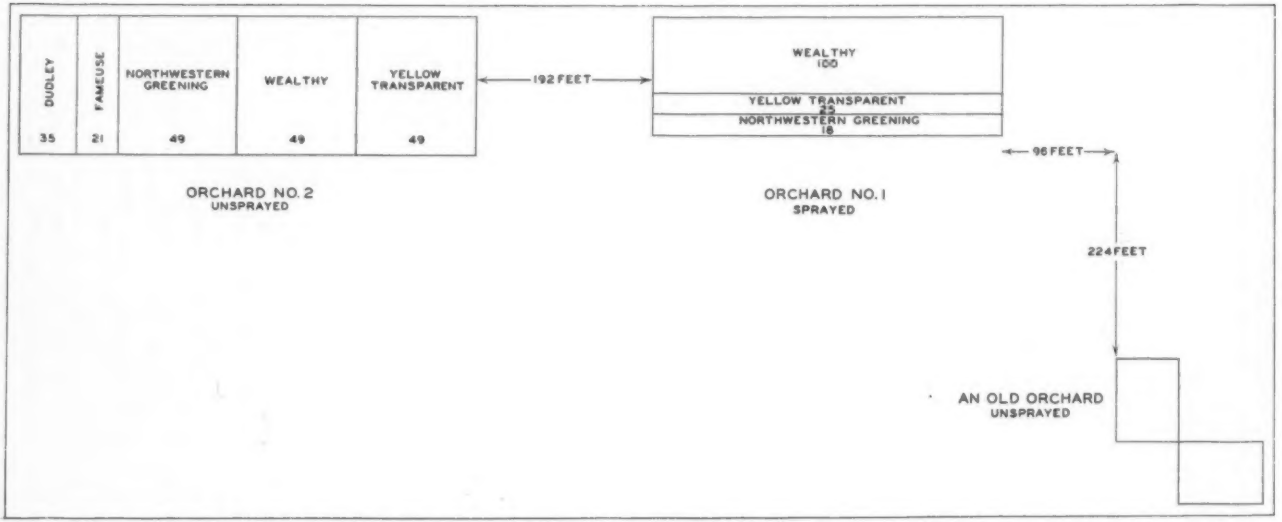

FIGURE 3.—Map of experimental orchards used in studies of the effects of eradicant spray treatments on the development of apple scab Madison, Wis., 1934-36. The figures in the several plots record the number of trees.

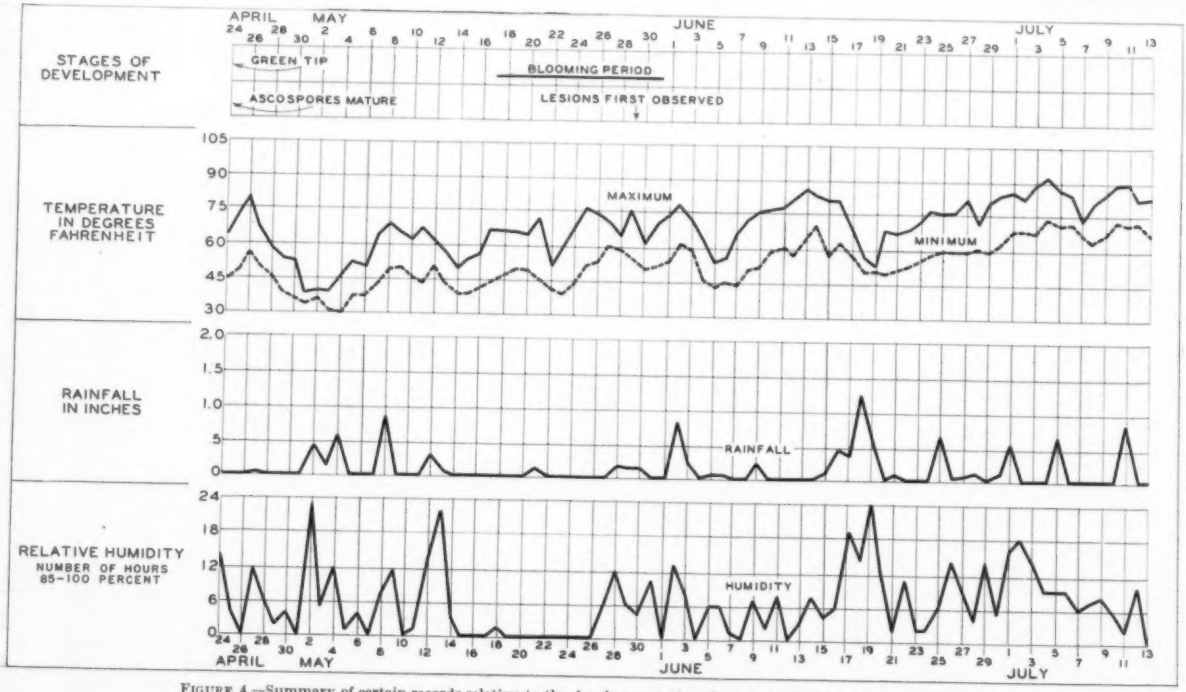

FIGURE 4.—Summary of certain records relating to the development of apple scab on Wealthy, Madison, Wis., 1935.

TABLE 4.—*Effects of fall spraying with eradicant fungicides on the development of apple scab in the following season, Madison, Wis., 1934–35 and 1935–36*

Year, variety, and date	Counts made on—	Orchard no. 1, sprayed [1]		Orchard no. 2, unsprayed		Decrease in disease in orchard 1 as compared with orchard 2
		Total units examined	Average lesions per unit	Total units examined	Average lesions per unit	
1934–35						
Wealthy:		*Number*	*Number*	*Number*	*Number*	*Percent*
May 31	Fruit spurs (leaf lesions)	500	0.078	250	1.708	96
June 24	Fruit	270	.42	113	4.50	91
June 29	Leaves of terminal shoots	283	.52	274	4.8	89
Northwestern Greening, June 29.	do	293	.72	282	18.0	96
1935–36						
Wealthy, June 2	do	2,575	.007	1,839	.648	99
Northwestern Greening, June 4.	do	1,219	.047	1,230	.832	94
Wealthy, June 6	do	3,042	.48	2,387	3.67	87
Northwestern Greening, July 9.	do	1,450	.71	1,464	4.88	85
Wealthy:						
July 10	Fruit	200	.075	200	.57	87
Sept. 2	do	1,334	1.93	718	7.79	75

[1] Orchard no. 1 sprayed in 3 subplots on Oct. 9, 1934, with formulas nos. 164, 191, and 205 of table 2; on Oct. 9, 1935, with formulas nos. 39, 143, and 152 (modified to contain zinc arsenite at ½ of 1 percent). No summer spray was applied to either orchard.

TABLE 5.—*Effects of fall spraying with eradicant fungicides on the development of apple scab in the following season, Madison, Wis., 1934–35 and 1935–36*

Year, variety, and date	Counts made on—	Orchard no. 1, sprayed [1]		Orchard no. 2, unsprayed	
		Total units examined	Units free of scab	Total units examined	Units free of scab
1934–35					
Wealthy:		*Number*	*Percent*	*Number*	*Percent*
June 24	Fruit	270	92	113	48
June 29	Terminal shoots (leaf lesions)	20	45	20	0
Northwestern Greening, June 29	do	20	50	20	0
Wealthy, Sept. 4	Fruit	320	89	(2)	-------
1935–36					
Wealthy, June 2	Terminal shoots (leaf lesions)	207	95	156	3
Northwestern Greening, June 4	do	100	66	100	1
Wealthy:					
June 8	Fruit	100	100	100	97
July 6	Terminal shoots (leaf lesions)	207	17	156	0
Northwestern Greening, July 9	do	100	11	100	0
Wealthy:					
July 10	Fruit	200	97	200	72
Sept. 2	do	1,334	45	718	1

[1] Orchard no. 1 sprayed in 3 subplots on Oct. 9, 1934, with formulas nos. 164, 191, and 205 of table 2; on Oct. 9, 1935, with formulas nos. 39, 143, and 152 (modified to contain zinc arsenite at ½ of 1 percent. No summer spray was applied to either orchard.

[2] No fruit available for counts.

EXPERIMENTS OF 1935–36

On October 9 all of orchard no. 1 was sprayed, formulas 39, 143, and 152 (modified to contain zinc arsenite, one-half of 1 percent) of table 2 being used on different subplots. Orchard no. 2 was un-

sprayed, except the Yellow Transparent block, which was given the same treatments as orchard no. 1 in order to increase the distance between that orchard and the source of ascosporic inoculum in the unsprayed orchard.

Conditions were generally favorable for production of perithecia of the fungus, although dry weather in the spring somewhat delayed ascospore discharge. Counts made by the usual method showed that in the plots treated, respectively, with formulas 39, 143, and 152, Wealthy leaves averaged 3, 4, and 9 perithecia per square inch of leaf surface examined. In the unsprayed orchard the leaves averaged 255 perithecia per square inch of leaf surface examined for Wealthy, 364 for Northwestern Greening. It appears, therefore, that production of perithecia in the sprayed orchard was reduced by approximately 98 percent.

Ascospores were ready for discharge by May 3. The first infection period occurred May 9–11 (fig. 5), and scab lesions were first observed May 29. The rains of late May and June permitted moderate infection. The development of the disease was sharply checked by hot, dry weather in July. Frequent periods of rain in August favored infection.

Beginning June 6, the seasonal development of the disease was followed on 20 tagged terminal shoots on as many trees in each orchard. The results are shown in figure 5.

Further comparative data on scab development in the sprayed and unsprayed orchards appear in tables 4 and 5. The results shown in table 4 indicate that through June and early July reduction of scab in the sprayed orchard was not less than 85 percent. Wealthy fruit of the sprayed orchard at harvest time showed a reduction of 75 percent. On June 2, 95 percent of the Wealthy terminal shoots in the sprayed orchard were free from scab lesions, against 3 percent in the unsprayed (table 5). On July 6, 17 percent were scab-free in the sprayed orchard, none in the unsprayed. On June 8 all the Wealthy fruit in the sprayed orchard was scab-free, against 97 percent in the unsprayed. At harvest 45 percent of the fruit of the sprayed orchard remained free from scab, against 1 percent in the unsprayed.

When this experiment was planned it was realized that the available orchards were too near together to preclude the probability of passage of a substantial ascosporic inoculum from the unsprayed to the sprayed. In view of this fact, the differences in scab development are the more striking.

In order to gain evidence concerning the possible influence of inoculum from the unsprayed orchard on disease development in the sprayed, the incidence of scab in the latter orchard was studied in relation to distance from the former (fig. 6). On July 6 data were taken on 50 Wealthy terminal shoots in each of the distance classes shown in figure 6. The results show that the incidence of infection diminished substantially with increased distance, an average of 12 lesions per terminal shoot occurring in the least distant class and 3 in the most distant. Conversely, the percent of terminal shoots free of scab increased with distance from 6 in the least distant class to 34 in the most distant.

Exact data on the distance to which ascospores of *Venturia inaequalis* may be carried in the air and the relations of distance from the source of the ascosporic inoculum to its concentration and to the

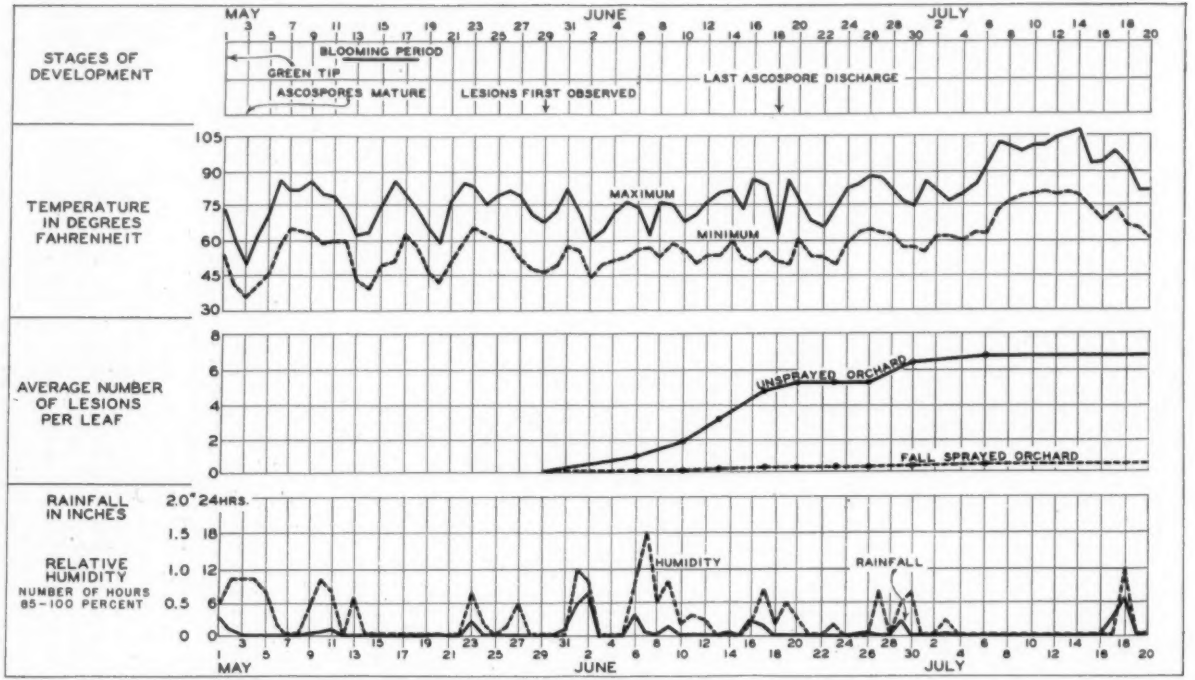

FIGURE 5.—Summary of certain records relating to the development of apple scab on Wealthy, Madison, Wis., 1936.

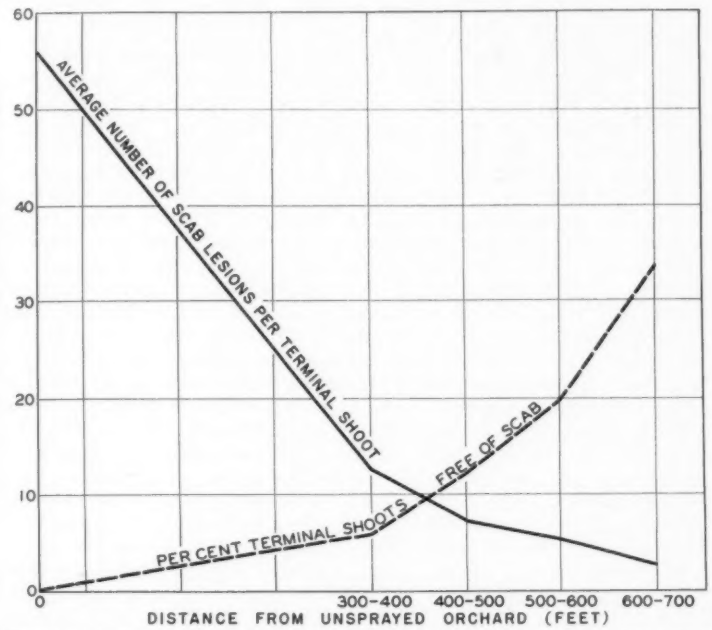

FIGURE 6.—Incidence of apple scab in the orchard (no. 1 of fig. 3) that received a fall treatment in relation to distance from the unsprayed orchard (no. 2), which contained an abundant ascosporic inoculum, Madison, Wis., 1936.

incidence of infection caused by it are meager. Undoubtedly the spores may be carried for great distances in the air (*23*). However, the limited evidence from the experiment that has just been reported, together with theoretical considerations and orchard observations, suggests that the concentration of this air-borne inoculum and the incidence of disease that it may cause diminish rapidly with the distance it is carried.

DISCUSSION

In the consideration of the results of the foregoing experiment, attention is invited to the fact that these fall treatments were not designed or expected to obviate the necessity of summer spraying. The summer sprays were omitted in order that effects of fall spraying might be studied more advantageously.

It should be observed that the seasonal conditions encountered in the course of this experiment did not favor heavy infection during the critical preblossom and blossom periods. Much more work under a wide range of conditions will be necessary to arrive at an adequate understanding of the relations of the quantitative level of the ascosporic inoculum to the epidemiology and control of the disease. The limited experimental data thus far obtained are in full accord with the evidence of earlier studies (*16*) in indicating that these relations are highly significant.

Seasonal conditions at Gays Mills,[6] Wis., in 1934 and 1935 afforded an opportunity for certain observations on the relations of a greatly reduced ascosporic inoculum to the seasonal development and control of scab. The extreme conditions of heat and drought in 1934, in conjunction with efficient summer spraying, so reduced the incidence of scab that perithecia of the pathogen were hard to find in certain large blocks of trees in the spring of 1935. The fungus, however, overwintered at a somewhat higher survival level in some situations where the spraying had been less thorough or the local environment more favorable, as in certain ravines. In 1935 conditions of moisture and temperature were very favorable for scab development, until late in the season. With a normal ascosporic inoculum a very severe and difficultly controllable epidemic of scab would have been expected. Actually, the disease development was moderate, and control was comparatively easy, especially in certain blocks where the ascosporic inoculum was known to have been very sparse. In one Dudley block in which the fungus was reduced to a very low survival level, only 49 percent of the unsprayed fruit showed scab at harvest; in a block of the same variety in which scab had been somewhat more prevalent the year before, 98 percent was scabbed. Unsprayed McIntosh in an orchard in which the fungus had overwintered at a very low survival level had only 88 percent of the fruit scabbed and 30 percent rendered unmarketable because of the disease. In this orchard a lime-sulphur program that began with the open-cluster spray reduced the percentage of scabby fruit to 2, and a like program beginning with the calyx application reduced it to 17, of which three-fourths could go into the U. S. No. 1 grade. This experience accords well with other data and with theoretical considerations in suggesting that a sufficient reduction in the ascosporic inoculum may be a potent factor in delaying and diminishing scab development and lessening the difficulty of its control by protectant sprays.

Chemical Treatment of Leaves on the Ground

In situations in which overwintering of *Venturia inaequalis* depends wholly or dominantly on production of the ascigerous stage, the period after leaf fall and before ascospore discharge is, obviously, a potentially vulnerable one. The fungus is then prostrate on the floor of the orchard. If it were as conspicuously visible as Canada thistle (*Cirsium arvense* (L.) Scop.), for example, would we continue to permit this dangerous and expensive pest to breed year after year in our orchards, essentially unmolested? Considerable progress has been made in the development of eradicant chemical treatments for use against weeds. Possibilities of adapting similar methods of chemical eradication to the control of apple scab and some other fungus diseases seem to deserve consideration.

Experiments with liquid applications of certain fertilizers that are known to have toxic properties and with certain weed killers have been initiated. In preliminary experiments in the spring of 1936, saturation of apple leaves as they lay on the ground by spraying with ammonium sulphate dissolved in water at the rate of 1 pound to the gallon killed the ascospores that were mature and prevented the maturation of others. Further results from this line of work are not yet available.

[6] Dr. J. A. Pinckard collaborated in this part of the investigation.

Present Status of the Work on Apple Scab

These studies on apple scab may be considered first in relation to the control of this disease, and second with reference to the broader question of the potentialities of eradicant fungicides for increased use against other plant diseases. The first of these aspects of the work is discussed in the following paragraphs, the second in a later section of this paper.

The problem relating directly to apple scab control has three major phases: (1) The development of means for sharply limiting the ascosporic inoculum; (2) studies of the effects of such limitation of the inoculum on the epidemiology and control of the disease; and (3) large-scale experiments to determine whether the procedures developed can be adapted to comprise a part of an improved program of scab control. Attention has thus far been devoted to the first and second phases. The results seem to have demonstrated that, under conditions such as were encountered in these experiments, (1) suitable applications of eradicant fungicides are capable of drastically reducing the ascosporic inoculum, and (2) such reduction greatly retards and ameliorates the development of the disease, especially in the critical period of primary and early secondary infection.

It is recognized that many obstacles must be overcome if fall spraying is to find a place in the apple scab control program. The most important appears to be the problem of host injury. Many of the formulas employed appear to be on the threshold of satisfactory fungicidal efficiency, without too much injury to the host. However, extensive tests, with due consideration to the effects of different environments and to varietal responses, will be necessary to establish the safety of such materials for use in orchard practice.

Other obstacles, such as early leaf fall under some conditions, difficulty of thorough coverage of the foliage of large trees, the inconvenience of spraying after harvest, etc., must also be considered. These difficulties vary much in importance with conditions in different regions. They do not seem to be insurmountable under ordinary Wisconsin conditions. It is scarcely to be expected that any control program will be adapted to all regions in which scab occurs.

Such eradicant chemical procedures as are here discussed can be established as a part of a sound program for apple scab control only if and when they justify themselves on the basis of extensive trial under orchard conditions. Much work remains to be done in developing methods and studying their effects before large-scale tests are warranted. Unless or until these methods stand the test of such trials, growers are advised to refrain from trying them.

The studies on apple scab are being continued.

EXPLORATORY STUDIES ON OTHER DISEASES

Though this investigation has been centered about apple scab, as an important disease that afforded opportunity and material for the work, its larger purpose has been to inquire into possibilities for wider application of the principle of eradication to plant disease control through the use of chemical agents. After it became evident that suitable applications of copper-lime-arsenic mixtures were capable of preventing the development of ascocarps of *Venturia inaequalis*, certain exploratory experiments were initiated to gain evidence

concerning their potentialities for limiting the development or the dispersal of an effective primary inoculum of certain other pathogenic fungi. A brief report of this work, which was necessarily limited in extent, follows. It seems unnecessary to burden this paper with a review, for each of the diseases dealt with, of the previous efforts in this direction, in which other materials, chiefly those adapted primarily for summer spraying, were employed.

BROWN ROT OF PLUM

On April 29, 1933, formulas CS1½+L¼+Cai¾+FO⅝ and CS1½+L⅜+Cai1+FO¾ (for explanation of symbols, see footnote 1, table 2) were applied to branches of *Prunus domestica* L. bearing persistent mummified fruits that had been attacked by the brown rot fungus, *Sclerotinia fructicola* (Wint.) Rehm, in the preceding year. The blossom buds had expanded but not separated in the clusters. Sprayed and unsprayed mummies, collected on May 5, were examined by a standardized procedure for occurrence of conidia. None were found on the sprayed mummies, whereas they occurred in abundance on the unsprayed. In a similar experiment in the following year formulas 10 and 12 of table 6 gave like results. No spray injury was observed to follow these treatments.

APPLE BLOTCH [7]

In 1934 and 1935 individual branches of three large Northwestern Greening apple trees bearing abundant twig lesions caused by the blotch fungus, *Phyllosticta solitaria* E. and E., were treated at Urbana, Ill., by means of hand sprayers.

In 1934 the trees were at the green-tip stage when the sprays were applied. Light showers occurred the night after the applications, followed by dry weather for several weeks. On June 2 and 14 twigs were collected at random from the treated and untreated branches and sent to Madison, where they were held for 5 days in a moist chamber at 20° C. to give the pycnidia further opportunity to mature. The percentage of lesions that bore any sporulating pycnidia was then determined by microscopic examination. Results from old lesions and those from new lesions and advancing margins of old ones were taken separately, the current year's advancing margins of an old lesion being counted as a new lesion.

In 1935 the experiment was repeated, with modifications. There were two dates of application, and the twigs were collected for examination on May 2.

A summary of the treatments and results for both years' experiments appears in table 6.

In 1934 the sprays were applied too late for maximal effectiveness. Nevertheless, formulas 1 and 2 seemed to prevent sporulation on about three-fourths of the old lesions (fig. 7, A, B). There was comparatively little reduction of sporulation on new lesions. Lime-sulphur and bordeaux treatments were comparatively ineffective. Formula 1 caused perceptible host injury; the others did not.

[7] The writers are indebted to Dr. H. W. Anderson of the Illinois Agricultural Experiment Station for his helpful cooperation in this part of the work and for permission to report the results, and to Fred Heaton of New Burnside, Ill., for making his orchard and spraying facilities available for the work of 1936.

TABLE 6.—*The effectiveness of certain spray treatments in suppressing sporulation of Phyllosticta solitaria in twig lesions of Northwestern Greening apple, Urbana, Ill., 1934 and 1935*

Date of application	Formula [1]	Total twigs observed	Old [2] lesions			New [2] lesions		
			Total	Bearing sporulating pycnidia		Total	Bearing sporulating pycnidia	
		Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1934	0. Untreated	10	15	15	100	16	16	100
Apr. 11	1. CS1½+L3⅛+Cal 1+FO3⅛	11	19	5	26	15	13	87
Do	2. CS1½+L3⅛+Cai¾+Zai¾+FO3¾	9	17	4	24	17	15	88
Do	3. CS1½+L3¼+Cai¾+Zai¾+FO7⅛	10	11	4	36	25	20	80
Do	4. CS1+L¼+Cai ½+Zai ½+FO1½	5	10	9	90	10	10	100
Do	5. CS1+L⅛+Cai ½+Zai ½+FO1½	7	14	10	72	24	24	100
Do	6. CS1+L⅛+Zai 1+FO1½	9	22	15	68	13	13	100
Do	7. CS1½+L3⅛+FO1½	10	19	14	74	19	19	100
Do	8. Lime-sulphur 1–7	9	22	21	96	13	13	100
1935	9. Untreated	12	49	49	100	17	12	71
Feb. 14	10. CS1½+L3⅛+Cai¾+Zai¾+FO3¾	6	42	0	0			
Apr. 8	do	15	45	12	27	12	4	33
Feb. 14	11. CS1½+L3⅛+Cai ¾+Copai ¾+FO3¾	7	68	2	3	3	0	0
Apr. 8	do	7	30	5	17	2	1	50
Feb. 14	12. CS1½+L¾+Cai¾+Zai¾+FO3¾	4	31	4	13	4	0	0
Apr. 8	do	10	30	26	87	4	0	0

[1] For explanation of symbols, see footnote 1, table 2.
[2] See text.

In 1935 the treatments applied February 14 were decidedly more effective than those of April 8. No sporulating pycnidia were found on old lesions that received the early application of treatment 10, and treatments 11 and 12 were nearly as effective, with the same timing.

Under the conditions of the experiment, few new lesions developed on the experimental branches. The data on these are too meager to offer more than a suggestion that there was some measure of limitation of sporulation on young lesions, especially by the earlier treatments.

In 1936 an experiment in which the sprays were applied by a power machine was performed at New Burnside, Ill. Applications were made March 4 on 10- to 15-year-old Duchess trees that bore abundant blotch lesions, especially on the fruit spurs. Each program of treatments was used on two or more trees. The experiment was planned primarily to gain evidence on the effects of the several programs on the incidence of blotch on fruit, but, owing to cold injury, practically no fruit was set. Furthermore, on account of the very dry season, there was no significant amount of new infection, even on unsprayed trees. Samples of twigs collected May 27 were examined microscopically on May 28 with reference to the percentage of pycnidia that bore spores. The results appear in table 7.

The examinations showed that 42 and 65 percent, respectively, of the pycnidia in the old lesions from the two plots (1, 2) that received no dormant spray contained spores; none in the old lesions of plots (3–8) that received the dormant treatment.

Where the dormant treatment was omitted (plots 1, 2), 100 percent of the pycnidia in the new lesions bore spores. In three of the four samples (plots 3, 5, 7, 8) that received both the dormant spray and the summer applications of bordeaux, only about half of the pycnidia

in the new lesions sporulated. In the samples that received the dormant treatment without the summer applications of bordeaux (plots 4, 6) the percentage of sporulating pycnidia was slightly higher.

TABLE 7.—*The effectiveness of certain spray treatments in suppressing sporulation of Phyllosticta solitaria in twig lesions of Duchess apple, New Burnside, Ill., 1936*

Plot no.	Formulas [1] applied Mar. 4, 1936	Summer treatment [2]	Type [3] and number of lesions observed		Total pycnidia observed	Pycnidia with spores	
					Number	*Number*	*Percent*
1	Untreated	Lead arsenate only	Old	5	57	24	42
			New	11	33	33	100
2	do	Bordeaux plus lead arsenate	Old	3	17	10	65
			New	7	24	24	100
3	CS1½+L3/6+Cal 3/4+Zal 3/4+FO3/4	do	Old	5	16	0	0
			New	19	55	27	49
4	do	Lead arsenate only	Old	5	20	0	0
			New	19	54	38	70
5	CS1+L3/6+Cal 3/4+Zal 3/4+FO3/4	Bordeaux plus lead arsenate	Old	--	--	--	--
			New	8	30	14	47
6	do	Lead arsenate only	Old	3	15	0	0
			New	7	27	16	59
7	CS1½+L3/6+Cal 1+Zal ½+FO3/4	Bordeaux plus lead arsenate	Old	5	19	0	0
			New	9	23	23	100
8	CS1+L1+Cal ½+Zal 1+FO3/4	do	Old	3	13	0	0
			New	16	41	20	49

[1] For explanation of symbols, see footnote 1, table 2.
[2] All plots were sprayed with flotation sulphur, 16–100, just before the blossoms opened, at petal fall, and 1 week later. Lead arsenate and hydrated lime, each 3–100, were added at petal fall. The bordeaux plots received applications of bordeaux, 4–6–100, plus lead arsenate, 4–100, at 2 and 3 weeks after petal fall. The other plots were treated at the same dates with lead arsenate and hydrated lime, each 4–100.
[3] See text.

None of the dormant spray treatments caused any perceptible host injury.

While the available data are very meager, they indicate that suitable applications of copper-lime-arsenic mixtures are capable of preventing sporulation in a high percentage of the pycnidia of the blotch pathogen, especially those borne in the old lesions. Whether these or similar treatments can be sufficiently perfected to become a practical measure for blotch control lies beyond the scope of the present investigation.

PEACH SCAB

On February 16, 1933, formulas 25, 30, and 31 of table 2 and bordeaux mixture, 4–2–50, were each applied at Madison by means of hand sprayers to twigs of *Amygdalus persica* L. bearing lesions caused by *Cladosporium carpophilum* Thüm. on the growth of the preceding year. On March 4 the twigs were collected and placed in a moist chamber at 22°–24° C. for 3 days to favor sporulation. They were then examined microscopically by a standardized procedure for incidence of conidia of the scab pathogen. The untreated lesions bore abundant conidia. The following percentage reductions in the number of spores borne on the treated lesions, based on the results from the untreated, were indicated: Formula 25, 100; formula 31, 99.2; formula 30, 93; bordeaux, 4–2–50, 60. Twigs sprayed with the copper-lime-arsenic mixtures showed some injury after growth was resumed, chiefly manifested as a roughening of the bark. The peach plant is well known to be very sensitive to copper injury. Lack of scab-infected peach trees precluded further experiments at Madison.

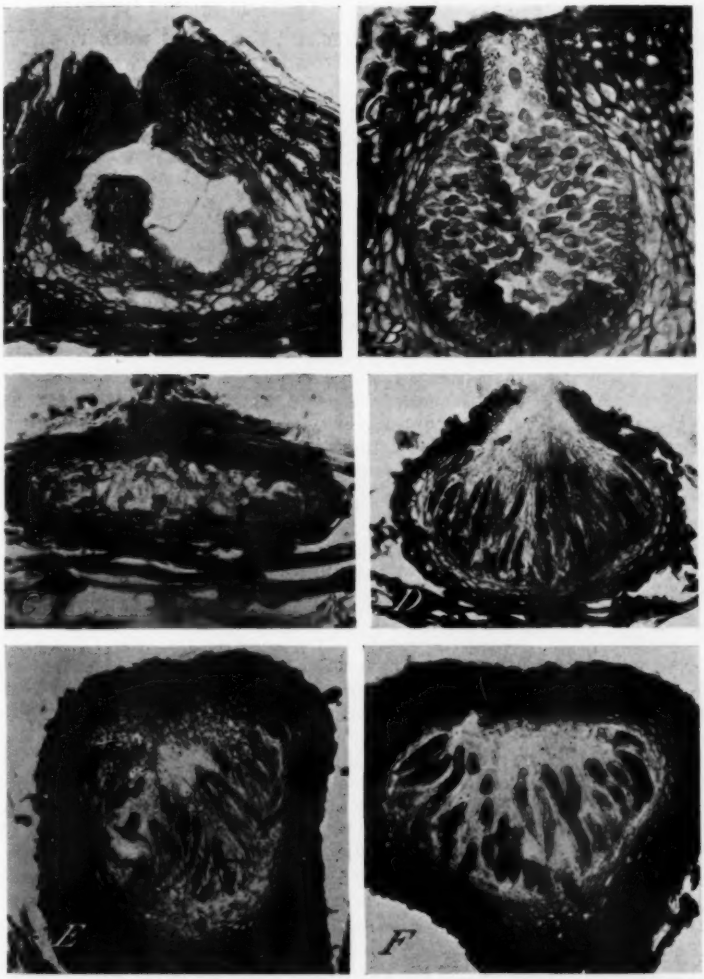

FIGURE 7.—Sections of fungal fruiting structures from fixed material, showing effects of eradicant spray treatments: *A*, Disorganized pycnidium of *Phyllosticta solitaria* 2 months after treatment with formula 2 of table 6; *B*, as *A* but untreated; *C*, disorganized ascocarp of *Mycosphaerella rubina* 17 days after treatment with a copper-lime-arsenic mixture; *D*, as *C* but untreated; *E*, disorganized ascocarp of *Dibotryon morbosum* 21 days after treatment with formula 12 of table 8; *F*, as *E* but untreated. All approximately × 250.

On March 15, 1935, three copper-lime-arsenic mixtures were applied to peach trees at Lexington, Ky.[8], by means of a power sprayer. The flower buds had already begun to show pink. Examination of twigs collected May 17 showed that the sprayed lesions bore about one-third to one-half as many conidia as the unsprayed. It seems probable that the lateness of application and the use of weaker mixtures may account in large measure for the difference between the Kentucky results and those obtained at Madison.

These data, though fragmentary and inconclusive, suggest possibilities of substantial reduction of sporulation of the peach scab fungus in twig lesions by means of suitable dormant spraying, and indicate that the copper-lime-arsenic sprays were far more effective than the bordeaux.

SPUR BLIGHT OF RASPBERRY

At Madison on May 15, 1933, several copper-lime-arsenic mixtures were applied by hand sprayers to canes of red raspberry (*Rubus strigosus* Michx.) affected by spur blight, caused by *Mycosphaerella rubina* (Pk.) Jacz. Microscopic examination of samples collected May 29 showed that perithecia on the sprayed canes were dead, whereas those on the unsprayed were in good condition. When fragments of moist bark bearing numerous perithecia were placed over clear agar in Petri dishes, there was abundant discharge of ascospores from the unsprayed material, none from the sprayed. Histological sections of sprayed and unsprayed perithecia collected June 1 are illustrated in figure 7, *C, D*.

PEAR SCAB

In a single small-scale experiment at Madison in 1933–34, pear (*Pyrus communis* L.) branches of an unknown variety bearing scabby leaves were treated in the fall with certain copper-lime-arsenic mixtures to test the effectiveness of the latter in suppressing production of perithecia of *Venturia pyrina* Aderh. Sprayed and unsprayed leaves were overwintered and examined for perithecia by the methods used in the similar studies on apple scab. The results, which will not be given in detail, were closely similar to those obtained in the apple scab work.

At Hood River, Oreg.[9], in 1934–35, certain copper-lime-arsenic mixtures were applied to branches of three large Anjou pear trees bearing twig lesions caused by *Venturia pyrina*. The same treatments were given on two dates, December 5 and March 18, so that one series of branches received only the earlier application, another only the later, and a third received both. Unsprayed branches served as controls. Because of circumstances beyond the control of the experimenters, these trees also received a dormant application of lime-sulphur.

Samples of twigs collected May 12 were sent to Madison, where they were examined microscopically for the percentage of lesions that bore conidia of the scab fungus. The results, which will not be given in detail, indicated that the more efficient treatments had reduced the percentage of sporulating lesions by approximately three-fourths

[8] Acknowledgment is made to Dr. W. D. Valleau of the Kentucky Agricultural Experiment Station for his kind cooperation in this experiment and permission to report the results.

[9] Acknowledgment is made to L. Childs of the Hood River Branch Experiment Station of Oregon and J. Kienholz of the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for their kind cooperation in this experiment and permission to report the results.

to nine-tenths. However, under the conditions of the experiment, there was more spray injury than could be tolerated commercially.

While the host injury caused in this experiment was distinctly discouraging, it should be noted that all the formulas used were comparatively low in lime. The fact that the conidial inoculum appeared to be substantially reduced by the formulas tested in this initial trial suggests possibilities of its more adequate suppression by better adapted sprays.

CHERRY LEAF SPOT

At Madison in 1933, branches of sour cherry (*Prunus cerasus* L.) abundantly affected by leaf spot, caused by *Coccomyces hiemalis* Higgins, were given fall applications of certain copper-lime-arsenic mixtures. Sprayed and unsprayed leaves were overwintered and examined for apothecia in the spring by the methods used in the similar studies on apple scab. The results, which will not be given in detail, were closely similar to those obtained in the studies on apple and pear scab.

BLACK KNOT OF CHOKECHERRY

In three series of experiments at Madison in 1933, certain sprays were applied by atomizers to cut twigs of chokecherry (*Prunus virginiana* L.) bearing lesions of black knot, caused by *Dibotryon morbosum* (Schw.) T. & S., to test their effectiveness in suppressing the production of ascospores of this pathogen. Two to ten twigs bearing living knots with abundant ascocarp initials or ascocarps were used per treatment. The cut ends of the twigs were kept in water, which was changed frequently. A summary of the treatments and results appears in table 8.

TABLE 8.—*Results of spraying cut twigs of chokecherry for the suppression of the ascigerous stage of the black knot fungus, Madison, Wis., 1933*

Series and date of application	Formula [1]	Ascospores present at stated dates	
Series 1, Feb. 1	1. Untreated	Yes	Feb. 15
	2. CS1+L (neut.)[2]+FO¼	Yes	
	3. CS1+L (neut.)+CaiI+FO½	No	
	4. CS1+ LI+CaiI+FO¾	No	
	5. CS1+L 1+Cai½+FO¾	No	
	6. CS¾+L ½+Cai½+FO¾	No	
Series 2, Feb. 16	7. Untreated	Yes	Feb. 25
	8. CS1+L ½+FO¾	Yes	
	9. CS1+L (neut.)+FO¼	Yes	
	10. CS1+L 1+Cai¾+FO¾	No	
	11. CS1+L 1+Cai ½+FO¾	No	
	12. CS1+L 1+Cai¼+FO½	No	
	13. CS1+L ½+Cai½+FO½	No	
	14. CS1+L ½+Cai¼+FO¾	No	
Series 3, Mar. 9	15. Untreated	Yes	Mar. 14
	16. CS1+L ½+Cai½+FO½	No	

[1] For explanation of symbols, see footnote 1 of table 2.
[2] Milk of lime was added in successive small amounts until the supernatant liquid, after agitation, no longer gave a positive potassium ferrocyanide test for copper.

On February 1, when the first series of sprays was applied, the asci of the black knot fungus were in an early stage of development. A few spores had been delimited, but none were mature. The twigs, after drying overnight in the laboratory, were wet with distilled water and held in a moist chamber for 1 week at 7° and for another at 12° C.

Abundant ascospores were developed on the unsprayed knots, and the ascocarps from this source were in good condition when examined on February 15. Abundant ascospores were also matured on the cankers sprayed with neutral bordeaux, but none on those sprayed with the copper-lime-arsenic mixtures. No asci were found in the material that received formula 4. Abundant asci were observed in the material that received formulas 3, 5, and 6, respectively; but they were abnormal, appeared to be degenerating, and bore no delimited spores. Fragments of knots from each treatment were moistened and placed in Petri dishes above agar for a test on spore discharge. Abundant ascospores were discharged from the untreated fragments. Discharge from the material that received the bordeaux treatment was abundant, but apparently less than from the untreated. No discharge occurred from fragments that had been treated with any of the copper-lime-arsenic mixtures.

The second series of sprays was applied February 16. The twigs, after drying overnight in the laboratory, were moistened and held in a moist chamber for 9 days at 16° C. The results of microscopic examinations and a test of ascospore discharge were similar to those of the first series. Abundant ascospores were developed on the untreated knots. Bordeaux failed to prevent abundant ascospore development and discharge. No mature ascospores were obtained from any of the knots that received the copper-lime-arsenic treatments. Figure 7, *E*, *F*, illustrates the condition of a typical perithecium that received treatment 12, in contrast with that of a normal ascocarp from an untreated lesion.

On March 9, when the third series was started, many of the ascocarps contained mature asci, and discharged ascospores freely when moistened. After the spray was applied the knots were allowed to dry out of doors. They were then wet with distilled water and placed in a moist chamber for 4 days at 16° C. On March 13 the unsprayed perithecia were normal in appearance, contained mature asci in abundance, and discharged ascospores freely. The asci in the treated ascocarps had degenerated, and no ascospores were discharged when fragments of the treated knots were moistened and placed over agar in Petri dishes.

THREAD BLIGHT OF FIG[10]

At Union, La., fig (*Ficus carica* L.) branches bearing abundant sclerotia of the thread blight fungus, *Corticium koleroga* (Cke.) v. Höhn. were sprayed January 25, 26 and again February 27, 28, 1935, with each bordeaux mixture (8–8–50 in the first application, 4–4–50 in the second) and certain copper-lime-arsenic formulas, respectively. The bordeaux treatments considerably reduced the amount of infection that developed in the following season, but did not satisfactorily control the disease. The two treatments with one of the copper-lime-arsenic preparations (formula 209 of table 2) gave efficient control. The disease developed abundantly on the unsprayed branches that served as checks. No spray injury was observed.

In 1936 the experiment was repeated with modifications. Two trees, about 12 years old and bearing abundant sclerotia, mycelia, and

[10] Acknowledgment is made to Dr. E. C. Tims and P. J. Mills, of the Louisiana Agricultural Experiment Station, for permission to make the following statement regarding their work on this disease.

basidiospores of the thread blight fungus, were sprayed January 9 and again February 26 with two copper-lime-arsenic formulas. Formula 108 of table 2 was used in both applications on tree no. 1, and formula 142 (with fish oil, one-half of 1 percent) of the same table was similarly applied to three-fourths of tree no. 2. The remainder of tree no. 2 served as a check. The disease was completely controlled on tree no. 1. A trace of infection (on two twigs) occurred on the sprayed portion of tree no. 2. The disease developed abundantly on the unsprayed part of tree no. 2. No spray injury was observed.

SEED TREATMENT EXPERIMENTS

The high eradicant fungicidal efficiency of copper-lime-arsenic mixtures and the ease with which their toxic properties can be varied through a wide range of control by modifications of formula suggested tests of their potentialities for use in seed treatments. The fact that these mixtures are capable of exercising toxic effects in an alkaline, neutral, or acid medium by means of soluble materials that diffuse from the spray or dust residues (*26*) suggested that they might find adaptation against seed- and soil-borne pathogens. About 200 dusts of good physical properties were prepared, usually by filtering the liquid preparations, drying the residues, and grinding in a mortar. The experimental work was centered about wheat (*Triticum aestivum* L.) bunt, caused by *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. In greenhouse experiments that have not yet been reported, certain of these copper-lime-arsenic dusts were somewhat more efficient than commercial copper carbonate or ethyl mercury phosphate dusts in controlling this disease in infested soil. In these tests, however, the copper-lime-arsenic dusts used were not capable of satisfactorily controlling bunt in cases of severe outbreaks resulting from a soil-borne inoculum. Dusts of the type under discussion clearly have substantial value for seed-treatment purposes. It remains for further investigation to determine whether they can advantageously be adapted to practical use.

DISCUSSION

The results of these studies as they relate to apple scab control have been discussed in an earlier section. It remains to consider them in relation to potentialities of increased use of eradicant chemical measures in the control of other diseases. This discussion will be introduced by the following brief survey of pertinent literature.

The first fungicides to find an important adaptation to plant disease control were of the eradicant type, and compounds of copper and arsenic, respectively, were among the first materials to be used successfully for this purpose. In his classic memoir on bunt of wheat, Prévost (*27*) reported experimental demonstration of the lethal action of copper sulphate and various other chemical agents on the spores of the bunt pathogen, and described a well-founded method for large-scale use of a copper sulphate solution for seed treatment. He reviewed previous work on seed treatments for bunt control, giving special attention to arsenous oxide, which had been used in England, and reporting it to be much inferior to copper sulphate in fungicidal action and other important qualities.

Washing dormant grapevines with a solution of iron sulphate and sulphuric acid to combat anthracnose was one of the earliest successful

adaptations of liquid fungicides to the treatment of plants for disease control (*29*). It was an eradicant procedure, and, if it had received more consideration, might have pointed the way to further substantial developments in this direction. However, the discovery of bordeaux mixture and the great development of dominantly protectant spraying that followed appear to have overshadowed this promising beginning in the evolution of methods for suppression of the primary inoculum of phytopathogenic fungi by eradicant chemical treatments.

The dominance of protectant spraying in the control of orchard diseases since the discovery of bordeaux mixture, the great achievements and present importance of protectant spraying, and the current status of eradicant measures in orchard disease control have been discussed by one of the writers (*15*).

In the rapid expansion of spraying methods that followed the introduction of bordeaux mixture, various treatments of dormant plants were tried more or less empirically against many diseases in the hope of suppressing the pathogens. In most cases, however, the results did not justify the treatments. While eradicant spraying has come into very successful use against certain diseases (e. g., peach leaf curl, caused by *Taphrina deformans* (Berk.) Tul.), it has continued to receive comparatively little attention.

Although arsenic is not usually regarded as an active fungicidal element, it has long been known that various arsenical compounds are toxic to fungi (*22*). The early work with arsenous oxide in seed treatments against bunt of wheat (*27*) has been mentioned. Knowles (*21*) reports an early attempt to combat dry rot of ships by the use of arsenical materials. Reference may be made to Horton and Salmon (*11*) and McCallan and Wilcoxon (*22*) for surveys of more recent literature on the fungicidal action of arsenical compounds. Of special interest in relation to the present paper are the work of Curtin (*2*) and Curtin and Thordarson (*3*) on the use of arsenites in wood preservation, that of Viala (*30*) and others on control of "esca" or apoplexy of the vine by a dormant application of an arsenical spray (chiefly sodium arsenite), and various experiments on the use of arsenical compounds in seed treatments (*22*) and in spraying plants in foliage (*5*, *7*, *8*, *9*, *11*, *28*) to combat certain diseases. McCallan and Wilcoxon (*22*) report that in their experiments on spore germination arsenic was toxic as arsenous acid, sodium arsenate ($Na_2HAsO_4.7H_2O$), and elementary powdered arsenic. They state that, in conformity with the results of Wollenweber (*34*), their data indicate "a great variation in sensitivity to arsenic among fungi", and suggest that, while arsenic may find increased use for fungicidal purposes, its adaptation will probably be confined to certain specific cases, such as they have noted. Working with a different technique, Curtin and Thordarson (*3*) found that zinc meta-arsenite was highly and rather uniformly toxic to the 14 species of fungi that they studied, and Palmiter and Keitt (*26*) report that calcium arsenites were highly toxic to all of the 8 species of phytopathogenic fungi included in their studies.

It seems unnecessary for the purposes of the present paper to discuss the rather extensive literature (*32*) dealing with the injurious or beneficial effects of arsenical compounds on seed plants, the accumulation of arsenic in the soil, its inactivation in the soil by chemical

reactions, its liberation from the soil in a gaseous state as a result of the activities of micro-organisms, and related subjects.

Additions of various arsenical compounds to bordeaux mixture for spraying plants in foliage have long been made for the purpose of insect control. Formerly, arsenites or materials containing them, such as paris green and london purple, were commonly used; but, in later years, these have largely been replaced by arsenates. It has long been recognized that the addition of these arsenical compounds may increase the fungicidal value of sprays. However, at the concentrations used in practice, such preparations have comparatively little eradicant fungicidal value, and are unsuited to the purposes of the present investigation.

The results that have been presented in the present paper and those of a correlated study on the toxicity of copper-lime-arsenic mixtures to certain phytophathogenic fungi, using toximetric cultural methods (*26*), show that suitable copper-lime-arsenic mixtures have powerful eradicant fungicidal properties, and that the toxicity of preparations of this type can be varied through a wide range by choice of the arsenical component, modifications in the amounts and proportions of the ingredients, and the use of amendments. Under the conditions employed in the toximetric experiments (*26*) the calcium arsenites studied showed a higher toxicity to the test fungi than copper sulphate, and the copper-lime-arsenite mixtures commonly showed a toxicity equal to or greater than the summation of the toxicities of their separate ingredients. Furthermore, it was shown that copper-lime-arsenic preparations liberate soluble materials that are capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues. Under like conditions, the copper-lime component (bordeaux mixture), when used alone, exercised little or no toxic action by means of diffused materials when the medium was alkaline or neutral, and very little in the acid range tested. These facts aid in explaining the great superiority of suitable copper-lime-arsenic preparations over bordeaux mixture in eradicant fungicidal power.

The studies reported herein show further that suitable copper-lime-arsenic mixtures were usually highly effective in either preventing sporulation of the various pathogens against which they were tested or else in killing or inactivating the spores before they were disseminated.

The dictum that a phytopathogenic fungus cannot be effectively combatted by spraying after it has become well established in the host tissues is rather widely accepted. Attention is invited to the fact that most pathogenic fungi that grow on or in plant tissues sporulate at or near the surface, or else their spores pass to the surface before they can be disseminated. The production and liberation of the primary inoculum is therefore to be regarded as a potentially vulnerable stage in the life history of many phytopathogenic fungi, and one against which surface treatments with suitable eradicant fungicides would seem to have much greater possibilities than have hitherto been generally recognized.

The studies thus far undertaken have been exploratory and comparatively limited. Much additional work remains to be done if eradicant chemical measures of the type under consideration are to find substantially increased practical adaptation in programs of soundly founded complementary procedures for the control of plant

diseases now combatted chiefly by protectant fungicides. The work reported herein was centered about copper-lime-arsenic preparations only because these seemed to be much the most promising materials tried. Further studies directed toward the development of better adapted materials seem desirable. Only a small beginning has been made on experimental studies of the effects of eradicant treatments, and the consequent limitation of primary inoculum, on epidemiology and control of the diseases concerned. Additional work in this undeveloped field and in the underlying fundamentals of epidemiology is highly essential to the evolution of soundly based control programs. Attempts at practical applications of the methods under investigation await further advances in studies of the eradicant methods and their effects.

The present methods of protectant spraying are the result of a prodigious amount of investigation and experience in many lands throughout a 50-year period. The problems of chemical eradication seem to be no less difficult than those of chemical protection. If the use of eradicant measures to complement protectant spraying is to be substantially advanced it will be only as the result of adequate experimentation. It is hoped that the evidence now available on the potentialities of eradicant chemical treatments for plant disease control may justify other workers in undertaking studies in this comparatively neglected field.

SUMMARY

Accomplishments and limitations of plant disease control by protectant spraying are discussed, and attention is called to the need for increased development of control programs of complementary procedures based on the principles of immunization and eradication as well as protection. A report is given of studies of potentialities for increased use of eradicant chemical measures for direct attack upon the pathogen, with the aim of reducing it to a survival level at which it can be more surely and economically controlled.

The studies on apple (*Malus sylvestris* Mill.) scab, caused by *Venturia inaequalis* (Cke.) Wint., were projected along three major lines: (1) Materials and methods for chemical eradication, (2) effects of eradicant procedures on epidemiology and control, and (3) large-scale orchard tests of the feasibility of adapting eradicant chemical measures to the practical control of the disease.

In small-scale experiments many materials were tested in a single spray treatment after harvest and before many leaves had fallen, with the aim of suppressing the ascosporic inoculum. Various mixtures of aqueous solution of copper sulphate, milk of lime, and certain arsenical compounds (chiefly calcium and zinc arsenites) were highly effective, often completely preventing production of ascospores in individual tests. The results from over 200 formulas are reported and discussed. Many of these mixtures caused severe host injury. Others showed high fungicidal effectiveness without having thus far caused serious injury to the host. Further studies are necessary to determine which formulas are best and whether they are safe enough for use in orchard practice.

In two seasons the epidemiology of apple scab was studied comparatively in two small orchards about 100 yards apart, one sprayed with copper-lime-arsenic mixtures in the fall and the other unsprayed.

Neither received summer sprays. Under the conditions encountered, scab development in the orchard that received the fall spray was strikingly retarded and reduced. Certain confirmatory orchard observations are reported.

A spring treatment of the fallen leaves with an ammonium sulphate solution killed the ascocarps of *Venturia inaequalis*. Other similar experiments are in progress.

The studies on apple scab, which are being continued, have not reached a stage to warrant large-scale trials on the feasibility of adapting the eradicant chemical methods here discussed to practical use in controlling this disease.

Small-scale exploratory tests of the potentialities of copper-lime-arsenic mixtures for suppressing primary inocula were made in relation to eight other phytopathogenic fungi. Fall spraying of pear (*Pyrus communis*) and cherry (*Prunus cerasus*) leaves infected, respectively, by *Venturia pyrina* and *Coccomyces hiemalis*, and dormant spraying of chokecherry (*Prunus virginiana*) and raspberry (*Rubus strigosus*) infected, respectively, by *Dibotryon morbosum* and *Mycosphaerella rubina* were highly effective in suppressing production of ascospores. Dormant spraying was highly effective in suppressing production of conidia of *Sclerotinia fructicola* on overwintered persistent mummified fruits of plum (*Prunus domestica*), and partly effective in suppressing production of pycnospores of *Phyllosticta solitaria* on apple and conidia of *Cladosporium carpophilum* and *Venturia pyrina* on peach (*Amygdalus persica*) and pear, respectively. It was highly effective against sclerotia, mycelia, and basidiospores of *Corticium koleroga*, appearing to give satisfactory control of the thread blight of fig (*Ficus carica*). In greenhouse trials in infested soil, copper-lime-arsenic dusts gave somewhat better control of wheat (*Triticum aestivum* Host) bunt, caused by *Tilletia tritici* and *T. levis*, than did commercial copper carbonate or ethyl mercury phosphate dusts.

The studies reported herein show: (1) That the toxicity of copper-lime-arsenic mixtures can be varied through a wide range of control by choice of the arsenical component, modifications in the amounts and proportions of the ingredients, and the use of amendments; (2) that these preparations liberate soluble materials that are capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues; (3) that suitable mixtures are highly effective against fungal fruiting structures at the surface, or near a permeable surface, of invaded tissues; and (4) that many phytopathogenic fungi are potentially vulnerable to attack by surface applications of eradicant fungicides.

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THE TOXICITY OF COPPER-LIME-ARSENIC MIXTURES TO CERTAIN PHYTOPATHOGENIC FUNGI GROWN ON MALT AGAR PLATES [1]

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INTRODUCTION

The work reported herein is part of a series of studies of potentialities for increased application of the principle of eradication to the control of certain types of plant disease through the use of fungicides. Keitt and associates (*2*, *3*, *5*, *6*)[2] have shown by means of spraying experiments that various copper-lime-arsenic mixtures possess strong eradicant fungicidal properties. The present paper reports the results of a comparative study of the toxicity of some of these mixtures and of their separate ingredients to certain representative phytopathogenic fungi grown in vitro (*4*). While it is recognized that such work cannot take the place of field experiments, the greater speed of operations and more accurate control of environmental conditions in the laboratory facilitate certain types of experimentation that materially assist in guiding the field work and interpreting its results.

TOXIMETRIC STUDIES

METHODS

The technique employed in this study is based on the widely used method of Schmitz et al. (*8*) for toximetric studies of wood preservatives.

The toxic materials were dissolved or suspended in water in 250-cubic centimeter flasks at twice the concentration desired in the toxic medium, and steamed at atmospheric pressure for 1 hour. When two or more chemicals entered into the toxic material, they were steamed separately in appropriate volumes of water. The nutrient medium (agar, 34 grams; Trommer's extract of malt, 50 grams; water, 1,000 cubic centimeters) was freshly prepared at twice the concentration used in the tests, and suitable quantities were autoclaved at 15 pounds' pressure for 30 minutes. After partial cooling, equal volumes of the medium and the solution or suspension of toxic material were mixed. When two or more chemicals entered into the toxic material they were introduced into the mixing flask in the following order: Copper or zinc sulphate, lime, the arsenical compound. The nutrient medium was added last, except in a few early experiments in which it was used to dilute the lime component. The results from these two procedures for adding the nutrient medium were so closely similar that they do not require separate treatment. The mixtures were shaken as each material was added. After thorough agitation to insure uniform suspension of solid materials, each mixture of agar and test material was

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[2] Reference is made by number (italic) to Literature Cited, p. 451.

poured in amounts of approximately 25 cubic centimeters into Petri plates, where after further agitation it was allowed to solidify. The temperature was regulated so that the toxic medium solidified very quickly after the plates were poured.

The mycelium of the test fungi for planting the plates was cut in disks 5 millimeters in diameter from malt-agar plate cultures of suitable age (5 to 10 days) to have developed a vigorous growth. Two plantings were made on each plate of toxic medium on a median line, each disk being about equally distant from the center and the edge of the plate. Control plates containing the suitably diluted medium, but no toxic material, were similarly planted in each test.

The plates were incubated in darkness at 20° C. for 2 weeks when the faster growing fungi, *Sclerotinia fructicola* (Wint.) Rehm. *Glomerella cingulata* (Stoneman) Sp. and con S., *Phyllosticta solitaria* E. and E., and *Physalospora obtusa* (Schw.) Cke., were used, and for 3 weeks when *Venturia inaequalis* (Cke.) Wint., *V. pyrina* Aderh., *Elsinoë veneta* (Burk.) Jenk., and *Cladosporium carpophilum* Thum. were the test organisms. If no growth appeared during this time the disks were transferred to malt agar slants, and if then no growth appeared after a period equal to that on the toxic medium the mycelium in the disks was considered dead.

Except as otherwise stated, the results are summarized (tables 1, 2, 3, 4) in terms of (1) the highest percentage concentration by weight of the toxic substance that in any case allowed growth of the test fungus on the plate, and (2) the lowest concentration that always killed the mycelium. At least two tests were made with each organism on each medium. Each test usually consisted of duplicate plates, with a total of four colonies, though as many as 5 plates and 10 colonies have been used. In general, the results of the replications agreed closely, though, as might be expected, those from some of the less toxic materials (e. g., diacalcium arsenate) showed more variation. Usually the concentration of the toxic materials was increased until the killing point was determined. However, some of the test materials did not kill at concentrations of 2 or 3 percent; and, since it is ordinarily impracticable to use such high dosages for spraying, the actual killing point of these preparations was not determined.

MATERIAL

Source and Purity of the Chemical Materials Used

The arsenical compounds used in this study, which were finely ground products obtained from several commercial companies, were analyzed[3] according to the methods of the Association of Official Agricultural Chemists (*1*). Results appear in the following paragraphs. The percent of moisture is based on the original air-dry samples. The water-soluble and total arsenic are expressed as percent As_2O_3 for the arsenites and As_2O_5 for the arsenates, based on the oven-dry (110° C.) samples. There was not sufficient difference in the results from different lots of the same compound to warrant distinctive designation of each lot in relation to the analyses.

Calcium arsenites.—While the calcium arsenites are designated as mono-, di-, and tri-, it is recognized that each probably contained

[3] Grateful acknowledgments are made to Prof. V. W. Meloche for supervising and to C. N. Clayton for making these determinations.

some admixture of the others. Each of the three samples of monocalcium arsenite used contained less than 1 percent of moisture. The water-soluble arsenic ranged from 23 to 28 percent, and the total arsenic from 70 to 74 percent. The two samples of dicalcium arsenite showed 2.7 and 4.5 percent of moisture, 8 and 15 percent of water-soluble arsenic, and 59 and 61 percent of total arsenic, respectively. The two samples of tricalcium arsenite showed 1.0 and 1.6 percent of moisture, 1.7 and 9.0 percent of water-soluble arsenic, and 55 and 54 percent of total arsenic, respectively.

Zinc arsenite.—All the zinc arsenite was obtained from the same source. It contained 0.4 percent of moisture, 0.6 percent of water-soluble arsenic, and 42 percent of total arsenic.

Copper arsenite.—The copper arsenite (c. p.), which was reported by the manufacturer to be in the cupric form, contained 2.2 percent of moisture, 2.0 percent of water-soluble arsenic, and 41 percent of total arsenic.

Paris green.—The paris green, which came from one source, contained 0.3 percent of moisture, 0.2 percent of water-soluble arsenic, and 57 percent of total arsenic.

Iron arsenite.—The iron arsenite (c. p.), which was reported by the manufacturer to be in the ferrous form, contained 5.6 percent of moisture, 3.0 percent of water-soluble arsenic, and 42 percent of total arsenic.

Calcium arsenates.—The dicalcium arsenate used contained 0.6 percent of moisture, 10 percent of water-soluble arsenic, and 54 percent of total arsenic as As_2O_5. The tricalcium arsenate used was not available when the arsenic determinations were made.

Other chemical materials.—Crystalline cupric sulphate ($CuSO_4.5H_2O$) of technical grade, c. p. crystalline zinc sulphate ($ZnSO_4.6H_2O$), and lump calcium oxide of U. S. P. grade were used.

Source of Test Fungi

The test fungi were obtained as follows: *Venturia inaequalis*, six monoconidial isolates (15, 17, 18, 22, 24, and 30) used and described by Palmiter (7); *V. pyrina*, a monoconidial isolate from a pear leaf at Madison, Wis.; *Cladosporium carpophilum*, a monoconidial isolate from peach twigs at Madison; *Phyllosticta solitaria*, isolated from apples from Indiana; *Elsinoë veneta*, from raspberry canes at Madison; *Glomerella cingulata*, a culture from H. W. Anderson, Urbana, Ill.; *Physalospora obtusa*, a monoconidial isolate from H. H. Foster, Madison; and *Sclerotinia fructicola*, a monoconidial isolate from cherry fruit at Sturgeon Bay, Wis.

EXPERIMENTAL RESULTS

TOXICITY OF THE SEPARATE INGREDIENTS OF CERTAIN COPPER-LIME-ARSENIC MIXTURES

Data on the toxicity of six arsenites, copper sulphate, and calcium oxide, respectively, to each of eight test fungi are shown in table 1. The toxicity of the arsenites varied through a wide range. The monocalcium preparation was the most toxic. The tricalcium arsenite was slightly less toxic. Paris green was less toxic than the calcium arsenites, but more toxic than copper arsenite. The zinc and iron

preparations were the least toxic of the arsenites tested, neither of them killing any of the test fungi except *Physalospora obtusa* at 2 percent, the highest concentration used.

The concentration of copper sulphate required to kill ranged from 3 to more than 10 times the lethal concentration of monocalcium arsenite, depending on the test fungus used.

The toxicity of calcium oxide varied much with the test fungi, one being killed at a concentration of 0.4 percent while three were able to withstand 2 percent.

No two test fungi reacted alike. *Physalospora obtusa* was the most sensitive, being killed by all the test materials used at concentrations less than 2 percent. *Glomerella cingulata* was the most resistant to copper sulphate, while *Sclerotinia fructicola* was the most resistant to the arsenites. Figure 1 shows the growth made by *Physalospora obtusa* on malt agar plates containing graduated concentrations of each of four different arsenites and of copper sulphate.

TABLE 1.—*Toxicity of the separate ingredients of certain copper-lime-arsenic mixtures to eight phytopathogenic fungi*

Toxic agent	Criteria	Critical concentrations [1] for—							
		Venturia inaequalis 17	*Venturia pyrina*	*Cladosporium carpophilum*	*Phyllosticta solitaria*	*Elsinoë veneta*	*Glomerella cingulata*	*Physalospora obtusa*	*Sclerotinia fructicola*
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Monocalcium arsenite	Growth	0.08	0.06	0.04	0.02	0.08	0.06	0.02	0.06
	Death	.10	.10	.06	.06	.10	.10	.04	.12
Tricalcium arsenite	Growth	.08	.08	.04	.02	.06	.06	.04	.06
	Death	.12	.12	.06	.08	.08	.10	.06	.14
Zinc arsenite	Growth	>2.00	>2.00	>2.00	>2.00	>2.00	>2.00	1.00	>2.00
	Death							1.50	
Iron arsenite	Growth	>2.00	>2.00	>2.00	>2.00	>2.00	>2.00	.40	>2.00
	Death							.50	
Copper arsenite	Growth	>2.00	>2.00	.50	.20	>2.00	1.00	.10	>2.00
	Death			1.00	>2.00		>2.00	.50	
Paris green	Growth	.50	>2.00	.10	.05	.50	.50	.05	.20
	Death	.60		.60	.20	2.00	.60	.10	1.00
Copper sulphate	Growth	.10	.20	.10	.15	.20	.30	.10	.80
	Death	.30	.45	.60	.20	.70	2.00	.60	1.00
Calcium oxide	Growth	.40	.40	.50	.30	.30	1.00	.40	.60
	Death	.80	.50	>2.00	1.00	.40	>2.00	.50	>2.00

[1] The figure shown for growth is the highest concentration tested at which growth occurred in any experiment; for death, the lowest that always killed. > indicates that the critical concentration was greater than the figure shown.

Data on the relative toxicities of each of four arsenites, two arsenates, copper sulphate, and calcium oxide to each of six monoconidia isolates of *Venturia inaequalis* are shown in table 2. The three calcium arsenites gave similar results and were consistently more toxic than copper sulphate. The tricalcium arsenate was the least toxic compound tested in this experiment, all of the isolates being able to grow at the highest concentrations used. The dicalcium arsenate was only slightly more toxic. The toxicity of paris green was intermediate between that of the calcium arsenites and the arsenates.

As pointed out by Palmiter (7), different isolates of *Venturia inaequalis* may show considerable variation in tolerance to toxic materials in the medium on which they are grown. Isolates 22 and 30 were more resistant to killing by the calcium arsenites than were the others. Isolates 22 and 17 were the more resistant ones to copper sulphate.

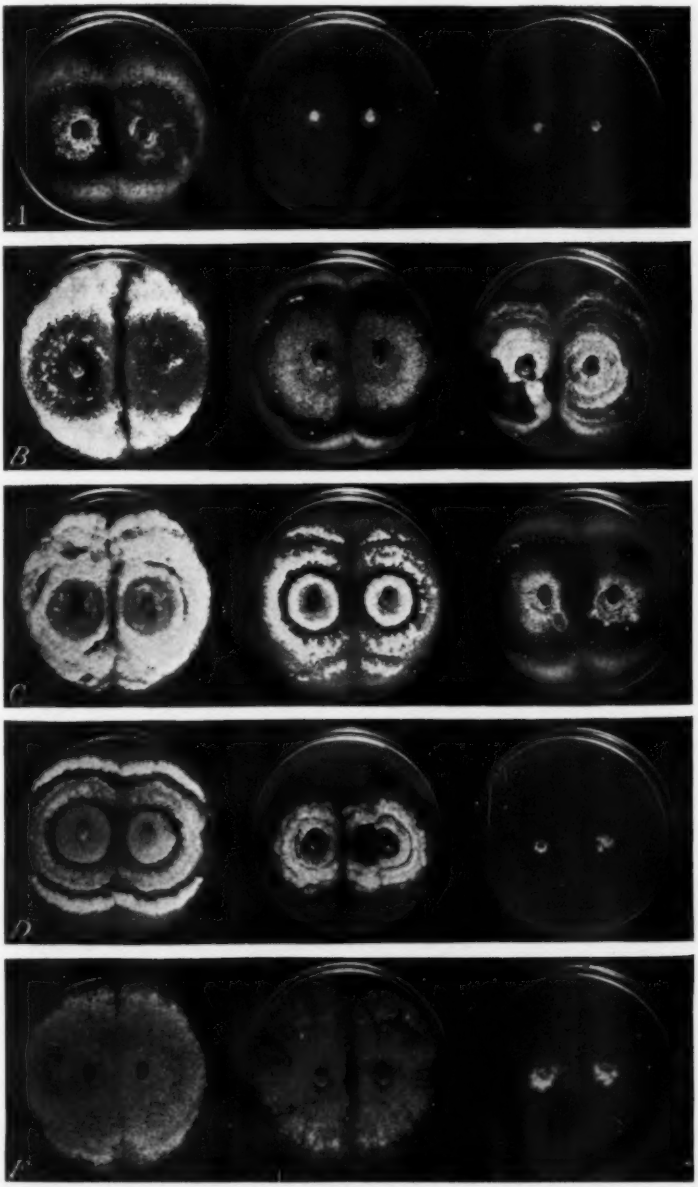

FIGURE 1.—Growth of *Physalospora obtusa* on malt agar plates containing from left to right 0.01, 0.025, and 0.05 percent, respectively, of: *A*, Monocalcium arsenite; *B*, zinc arsenite; *C*, iron arsenite; *D*, copper arsenite; *E*, copper sulphate.

TOXICITY OF CERTAIN COPPER-LIME-ARSENIC MIXTURES

Data on the toxicity of certain copper-lime-arsenic mixtures and of their copper-lime component, used alone, are shown in table 3. The copper-lime-monocalcium arsenite preparation was the most toxic of the mixtures studied, *Sclerotina fructicola* being the only test fungus that was not killed by 10 percent of the reference concentration. The similar tricalcium arsenite preparation was distinctly less toxic. The paris green mixture was more toxic than the tricalcium arsenite preparation against certain test fungi and less toxic against others. In all cases it was more toxic than the zinc, iron, or copper arsenite mixtures. The copper-lime component, used alone, was relatively low in toxicity, most of the test fungi surviving at the highest concentration tried.

TABLE 2.—*Toxicity of the separate ingredients of certain copper-lime-arsenic mixtures to 6 isolates of Venturia inaequalis*

Toxic agent	Criteria	Critical concentrations [1] for isolate—					
		15	17	18	22	24	30
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Monocalcium arsenite	Growth	0.08	0.08	0.08	0.12	0.08	0.10
	Death	.12	.12	.12	.18	.12	.14
Dicalcium arsenite	Growth	.08	.08	.08	.10	.08	.08
	Death	.12	.12	.10	.12	.12	.14
Tricalcium arsenite	Growth	.08	.08	.10	.10	.08	.08
	Death	.10	.12	.12	.16	.12	.12
Paris green	Growth	.30	.50	.30	.50	.30	.30
	Death	.50	.60	>.30	1.50	>.30	>.30
Dicalcium arsenate	Growth	1.00	.60	1.00	1.80	1.00	.50
	Death	3.00	>3.00	>2.00	>3.00	>2.00	>2.00
Tricalcium arsenate	Growth	>3.00	>3.00	>2.00	>3.00	>2.00	.....
	Death	.....	.....	.....	.....	.....	.....
Copper sulphate	Growth	.08	.10	.12	.12	.10	.14
	Death	.14	.30	.18	.40	.16	.18
Calcium oxide	Growth	>.50	.40	>.50	>.50	>.50	.10
	Death	.....	.80	.....	.....	.....	.50

[1] See footnote 1, table 1.

TABLE 3.—*Toxicity of certain copper-lime-arsenic mixtures to 8 phytopathogenic fungi*

Formulas at reference concentrations [1] in grams per 100 cubic centimeters	Criteria	Critical concentrations [1 2] of reference formulas for—							
		Venturia inaequalis 17	*Venturia pyrina*	*Cladosporium carpophilum*	*Phyllosticta solitaria*	*Elsinoë veneta*	*Glomerella cingulata*	*Physalospora obtusa*	*Sclerotinia fructicola*
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A [3]	Growth	10	20	10	10	10	>100	20	20
	Death	100	>100	>100	100	>100	>100	>100	>100
A+monocalcium arsenite, 0.5	Growth	5	5	2	5	5	5	2	5
	Death	10	10	10	10	10	10	5	20
A+tricalcium arsenite, 0.5	Growth	5	10	5	5	5	5	2	10
	Death	>10	>100	40	10	10	100	5	>100
A+zinc arsenite, 1.0	Growth	20	20	10	10	10	10	10	10
	Death	>100	>100	>100	60	>100	>100	40	>100
A+iron arsenite, 0.5	Growth	60	60	5	5	20	10	5	20
	Death	>100	>100	100	100	100	>100	20	>100
A+copper arsenite, 0.5	Growth	60	5	5	5	5	10	5	10
	Death	100	>100	100	60	60	>100	20	>100
A+paris green	Growth	10	10	5	5	5	5	5	5
	Death	60	60	60	10	20	40	10	>100

[1] The formulas are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.
[2] See footnote 1, table 1.
[3] A = the copper-lime component: $CuSO_4 \cdot 5H_2O$, 0.75 grams, plus calcium oxide, 0.5 grams, per 100 cubic centimeters.

In tests with *Venturia inaequalis* (table 4), the copper-lime-monocalcium arsenite mixture was more toxic than the similar dicalcium and tricalcium arsenite preparations, and equally toxic to all six isolates used. The similar dicalcium and tricalcium arsenate mixtures showed little increase in toxicity over the preparation containing only the copper sulphate and lime. When zinc sulphate was substituted for the copper sulphate, a slight decrease in toxicity resulted.

Whereas the several isolates of *Venturia inaequalis* differed considerably from one another in their reactions to the separate ingredients of the copper-lime-arsenic mixtures (table 2), they were rather uniform in their response to the mixtures (table 4).

The failure of certain fungi to grow on media containing comparatively high concentrations of lime suggested a study of the tolerance of high alkalinity by the test organisms. Experiments were therefore performed to determine the optimal pH value for growth of the test fungi and the upper pH limits at which they could be expected to make satisfactory growth for these tests. Varying amounts of sodium hydroxide were added to the malt extract agar to give the desired range of pH. On the basis of diameter of colony, *Glomerella cingulata* and *Elsinoe veneta* made their best growth when the initial pH value of the medium was 8.0 to 8.7, while the other six fungi grew best when the medium was adjusted in the range of pH 5.3 to 6.7. All eight fungi were able to make satisfactory growth for these tests when the medium had an initial pH value of 8.5.

When the ingredients of the mixtures were used separately in the medium all the toxic media except those made with calcium oxide gave pH readings below 8.5. The medium containing 0.5 percent of the calcium oxide, used alone, was too alkaline for satisfactory growth of the test fungi.

TABLE 4.—*Toxicity of certain copper- or zinc-lime-arsenic mixtures to 6 isolates of Venturia inaequalis*

Formulas at reference concentrations[1] in grams per 100 cubic centimeters	Criteria	Critical concentrations[1 2] of reference formulas for isolate—					
		15	17	18	22	24	30
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A[3]	Growth	20	10	10	20	20	20
	Death	>100	100	>100	>100	>100	>100
A+monocalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	10	10	10	10	10
A+dicalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	10	>10	>10	>10	>10
A+tricalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	>10	10	>10	>10	>10
A+dicalcium arsenate, 1.0	Growth	5	5	------	10	------	------
	Death	>100	>100	------	>100	------	------
A+tricalcium arsenate, 1.0	Growth	10	5	------	10	------	------
	Death	100	100	------	100	------	------
B[4]	Growth	20	100	20	5	20	100
	Death	>100	>100	>100	>100	100	------
B+tricalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	>10	>10	>10	>10	>10

[1] The formulas are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.

[2] See footnote 1, table 1.

[3] A=the copper-lime component: $CuSO_4.5H_2O$, 0.75 gram, plus calcium oxide, 0.5 gram, per 100 cubic centimeters.

[4] B=the zinc-lime component: $ZnSO_4.6H_2O$, 0.75 gram, plus calcium oxide, 0.5 gram. per 100 cubic centimeters.

When the copper-lime or copper-lime-arsenic mixtures were used the reaction was favorable for growth of all the test organisms, except when the amount required to kill approached 100 percent of the reference formulas. For example, the copper-sulphate-lime-monocalcium arsenite preparation killed all the test fungi at or below 20 percent of its reference formula (table 3), and at the 20-percent concentration had an initial pH value of approximately 8.1. However, mixtures containing some of the less toxic arsenites did not kill until the concentration approached 100 percent of the reference formulae. At the reference concentrations the media had reactions in the range of pH 9 to 10.

RELATION OF THE TOXICITY OF CERTAIN COPPER-LIME-ARSENITE MIXTURES TO THE SUMMATION OF THE TOXICITIES OF THEIR SEPARATE INGREDIENTS

The materials used for making certain copper-lime-arsenite mixtures were studied separately and in the mixtures at reference concentrations suitable for comparing the summation of the indicated toxicities of the separate ingredients of a given preparation with that of the mixture (table 5). Since the copper-lime-monocalcium arsenite mixture was the most toxic material used, killing the test organism, *Physalospora obtusa*, at 5 percent of its reference concentration, its toxicity was given a value of 100 and made the standard from which to compute the relative toxicities of the other materials (5÷lethal concentration of the given material×100).

TABLE 5.—*Increased toxicity of certain copper-lime-arsenite mixtures to Physalospora obtusa as compared with the summation of the toxicities of their separate ingredients*

Formulas or separate ingredients at reference concentrations [1] in grams per 100 cubic centimeters	Concentration [1] required to kill	Relative toxicity [2]	Formulas or separate ingredients at reference concentrations [1] in grams per 100 cubic centimeters	Concentration [1] required to kill	Relative toxicity [2]
	Percent			*Percent*	
Copper sulphate, 0.75	80	6.2	Iron arsenite, 0.5	100	5.0
Calcium oxide, 0.5	100	5.0	A+iron arsenite, 0.5	20	25.0
A [3]	100	5.0	Zinc arsenite, 1.0	150	3.3
Monocalcium arsenite, 0.5	8	62.5	A+zinc arsenite, 1.0	40	12.4
A+monocalcium arsenite, 0.5	5	100.0	Paris green, 1.0	100	5.0
Copper arsenite, 0.5	100	5.0	A+Paris green, 1.0	10	50.0
A+copper arsenite, 0.5	20	25.0			

[1] The formulas or separate ingredients are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.
[2] Relative toxicity=5÷killing concentration × 100 (see text).
[3] A=the copper-lime component: $CuSO_4.5H_2O$, 0.75 gram, plus calcium oxide, 0.5, per 100 cubic centimeters.

The results presented in table 5 show that each of the copper-lime-arsenite mixtures tested in this experiment, except the one containing zinc arsenite, had a greater toxicity than would be indicated by adding the toxic values of the ingredients that entered into its composition. Copper sulphate, calcium oxide, and monocalcium arsenite, used separately, had relative toxicities of 6.2, 5, and 62.5, respectively, a total of 73.7, as compared with a value of 100 when these same materials were mixed. The zinc, copper, and iron arsenite mixtures showed similar increases in toxicity over the summation of the toxicities of the separate ingredients. Figure 2 shows the very meager growth

made by *Physalospora obtusa* when the copper-lime component and the zinc arsenite were mixed (*A*), as compared with a much increased growth when they were used separately (*B*, *C*). Paris green, used alone, had a relative toxicity of 5, which added to 6.2 for the copper sulphate and 5 for the lime makes a total of 16.2, against a value of 50 when the materials were mixed. If the relative toxicity of the mixture of the copper sulphate and lime, 5, is taken instead of the

FIGURE 2.—Comparative toxicity to *Physalospora obtusa* of a copper-lime-arsenite mixture and its copper, lime, and arsenical ingredients, respectively: *A*, Copper sulphate 0.015 percent plus lime 0.01 percent plus zinc arsenite 0.01 percent; *B*, copper sulphate 0.015 percent plus lime 0.01 percent; *C*, zinc arsenite 0.01 percent.

relative toxicities of its separate ingredients, 6.2 and 5, the increased toxicity of the copper-lime-arsenite mixtures over the summation of the toxicities of their ingredients is still more notable.

The amount of increased toxicity of the copper-lime-arsenite mixture over that of its separate ingredients varies greatly with the test

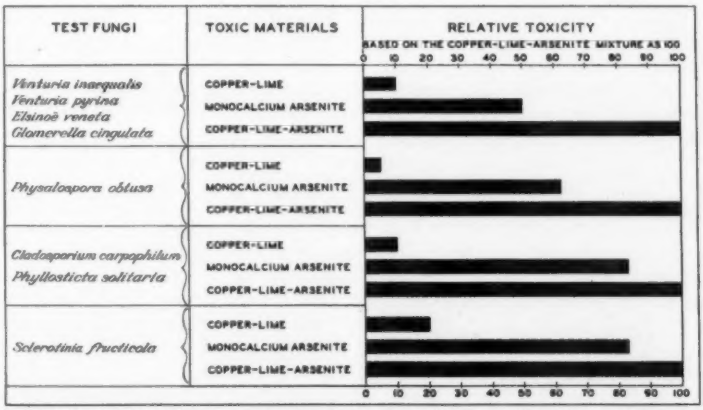

FIGURE 3.—Increased toxicity of a copper sulphate-lime-monocalcium arsenite mixture over the summation of the toxicities of its copper, lime, and arsenical ingredients. The data that apply to more than one test organism are averaged. The composition of the copper-lime-arsenite mixture and the method of computing the relative toxicities are shown in table 5.

fungi, as shown in figure 3. *Physalospora obtusa*, the test organism in the experiment discussed in the preceding paragraph, being quite sensitive, is killed by relatively small amounts of calcium arsenite. Even so, it showed an increased toxicity of the mixture over the summation of the toxicities of the ingredients, amounting to more than 50 percent. With *Venturia inaequalis*, *V. pyrina*, *Elsinoë veneta*, and

Glomerella cingulata, which can tolerate higher concentrations of the separate ingredients, the copper-lime-arsenite mixture had a toxicity 66 percent greater than the added toxicities of the copper, lime, and the monocalcium arsenite ingredients. With *Cladosporium carpophilum* and *Phyllosticta solitaria* only a slight increase in toxicity of the mixture over the added toxicities of its ingredients was indicated; with *Sclerotinia fructicola*, none. In the last case the sum of the indicated toxic values of the separate ingredients was 103. However, this was because the lethal concentration of the copper-lime component was not determined (table 3), the 100-percent concentration being treated in the computation as if it were lethal. Since only *Venturia inaequalis* and *Phyllosticta solitaria* were actually killed by the copper-lime component, the increased toxicity of the mixture to the other test fungi is really greater than that shown in figure 3.

COMPARATIVE EFFECTIVENESS OF COPPER-LIME-ARSENIC MIXTURES AND OF THEIR SEPARATE INGREDIENTS IN SUPPRESSING FUNGAL DEVELOPMENT IN THE VICINITY OF THEIR SOLID RESIDUES

Results of the foregoing experiments and work reported elsewhere (*5*) indicate that copper-lime-arsenic preparations contain or liberate soluble materials capable of exercising fungicidal action at greater or less distance from their solid residues. The following limited studies were undertaken to gain some further evidence regarding the comparative effectiveness of certain copper-lime-arsenic mixtures and of their separate ingredients in suppressing the development of a test fungus in the surrounding medium by means of diffused toxic materials.

The medium used was made of agar, 17 grams; Trommer's malt extract, 5 grams; and water, 1,000 cubic centimeters; adjusted to pH 7. The toxic materials tested were chosen from dusts prepared for seed-treatment experiments, which will be reported elsewhere. One part by weight of each dust material was mixed with 3 parts of the agar medium, and the resulting suspension was allowed to solidify into a slab of standard thickness after thorough agitation to facilitate uniformity of distribution of the test substance. Disks of the toxic agar suspension 5 millimeters in diameter were placed in Petri plates containing uniform amounts of the malt agar, which had been seeded by a standardized procedure with conidia of the test fungus, *Gibberella saubinetii* (Mont.) Sacc. The disks were inserted in the number and positions shown in figure 4, just before the agar solidified. In control plates no toxic disks were inserted. After incubation for 4 days at 20° C., the plates showed a uniform development of the fungus, except in areas about the disks, where it had been killed or inhibited by the diffused toxic materials. The size of the clear areas about the disks is, therefore, an approximate index of the comparative efficiency of the test materials under the experimental conditions. Illustrative results are shown in figure 4. The fungus grew over the entire surface of the control plates (*A*). It grew up to the edge of the copper-lime disks, though it was distinctly inhibited in a narrow zone (*B*). Zinc arsenite was lethal through a comparatively narrow zone (*C*), while monocalcium arsenite was lethal through a zone about twice as wide (*D*). The copper-lime-arsenic preparations were effective through a substantially wider zone than were the arsenical materials that, respectively, entered into their composition (*E*, *F*). Attention

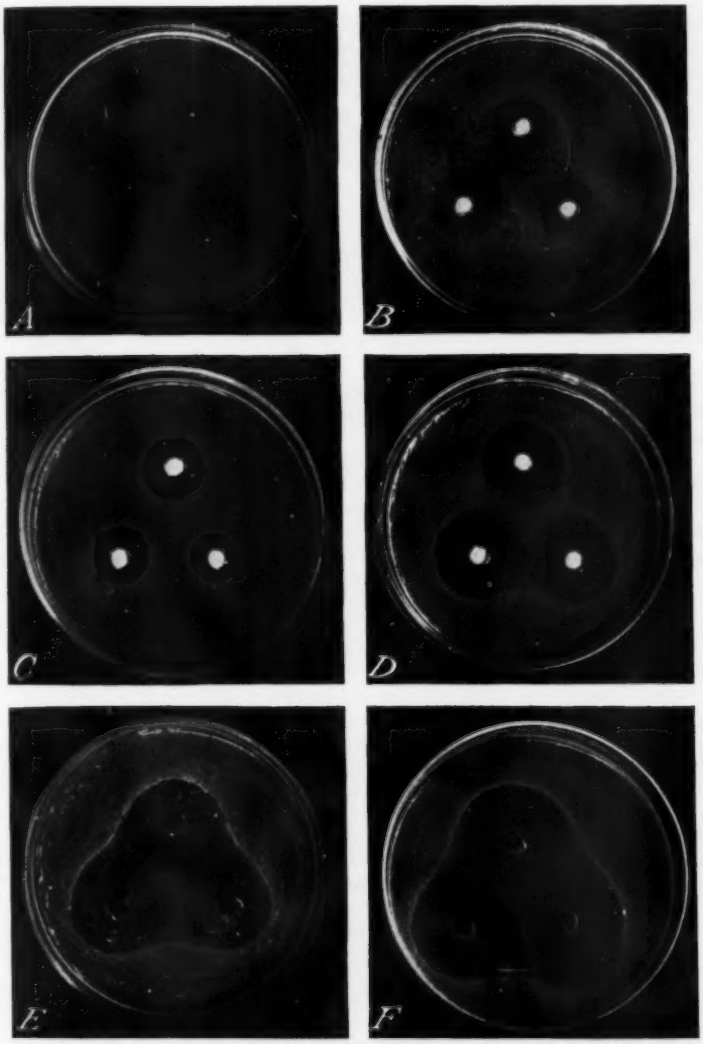

FIGURE 4.—Comparative effectiveness of certain copper-lime-arsenic mixtures and of their separate ingredients in suppressing the development of *Gibberella saubinetii* in a malt-agar medium by means of diffused toxic materials: *A*, Control; *B*, copper-lime mixture (copper sulphate, 1 percent, lime, ⅛ percent); *C*, zinc arsenite; *D*, monocalcium arsenite; *E*, copper-lime-arsenic mixture (copper sulphate, 1 percent, lime, ⅛ percent, monocalcium arsenite, ½ percent); *F*, copper-lime-arsenic mixture (copper sulphate, 1 percent, lime, ¼ percent, monocalcium arsenite, ½ percent). The mixtures were prepared as for spraying, filtered, dried, and ground. (See text.)

is invited to the fact that in these experiments the several test materials were compared in equal amounts by weight, rather than in the proportions in which they were used in the mixtures.

In similar experiments the initial reaction of the medium was varied. The copper-lime-arsenic preparations used were capable of exerting a substantial toxic action by means of materials that diffused from the solid residues into the surrounding medium, whether its initial reaction was alkaline, neutral, or acid. Similar toxic action by the copper-lime ingredient was much less at all reactions tried, being practically nil in the alkaline range, slight at neutrality, and somewhat increased in the acid range.

The properties just discussed account in part for the high eradicant effectiveness of copper-lime-arsenic mixtures against fungal fruiting structures, and suggest possible adaptations of these materials for combating seed- or soil-borne pathogens.

DISCUSSION

The literature on the toxicity and fungicidal use of arsenical compounds and copper-lime-arsenic mixtures has been surveyed by Keitt and Palmiter (*5*).

In confirmation of the conclusions of Keitt and Palmiter (*5*), the results of the toximetric experiments reported herein show that suitable copper-lime-arsenic mixtures have powerful fungicidal properties, and that their toxicity can be varied through a wide range by modifications of formula. A striking feature of these studies is the high and comparatively uniform toxicity of certain copper-lime-arsenic mixtures to all the test fungi used.

The fact that the toxicity of the copper-lime-arsenite mixtures studied was usually greater than the summation of the toxicities of their separate ingredients is of interest, both in relation to their superior fungicidal effectiveness in spraying experiments (*5*) and for theoretical considerations. The reasons for this increased toxicity of the mixtures have not been investigated. It is noteworthy, however, that these mixtures contain both a toxic anion and a toxic cation. Furthermore, there are manifold possibilities of changes in toxicity incident to chemical reactions and physiochemical changes in such complex preparations.

The ability of suitable copper-lime-arsenic mixtures to liberate soluble materials capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues is an important factor in their effectiveness as fungicides (*5*).

SUMMARY

The toxicity of certain copper-lime-arsenic mixtures and of their separate ingredients was studied by a modification of the method of Schmitz et al., using as test organisms *Venturia inaequalis*, *V. pyrina*, *Cladosporium carpophilum*, *Phyllosticta solitaria*, *Elsinoë veneta*, *Glomerella cingulata*, *Physalospora obtusa*, and *Sclerotinia fructicola*.

Suitable copper-lime-arsenic mixtures were highly toxic to all the test fungi. The toxicity of such mixtures could be varied through a wide range by modifications of formula.

Copper sulphate-lime mixtures had relatively low toxicity.

The toxicity of the arsenical compounds tested varied through a wide range. Monocalcium arsenite was the most toxic, usually being from 3 to more than 10 times as toxic as copper sulphate, depending on the test fungus used. Tricalcium arsenite was slightly less toxic. Zinc and iron arsenites were comparatively low in toxicity, while paris green and copper arsenite were intermediate. Tricalcium arsenate was the least toxic arsenical compound tested, and dicalcium arsenate was only slightly more toxic.

Six monoconidial isolates of *Venturia inaequalis* showed about as much variation in reaction to copper sulphate and certain arsenites, respectively, as did species of six other genera.

The toxicity of the copper-lime-arsenite mixtures studied usually exceeded the summation of the toxicities of their separate ingredients.

It is shown in tests with *Gibberella saubinetii* that suitable copper-lime-arsenic mixtures liberate soluble materials capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues.

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RESTORATION OF VIRULENCE OF ATTENUATED CURLY TOP VIRUS BY PASSAGE THROUGH SUSCEPTIBLE PLANTS [1]

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INTRODUCTION

The sugar-beet curly top virus, after passage through *Chenopodium murale* L., was shown by Carsner [3] to produce mild effects when subsequently inoculated into sugar-beet plants. This indicated change enough to warrant the designation "attenuated virus." The writer has shown that other plants may function similarly [4] and that virulent curly top virus, after it has been attenuated by passage through *C. murale*, may be restored to its original condition of virulence, or nearly so, by a single passage through chickweed, *Stellaria media* (L.) Cyr.[5]

In nature, restoration of virulence by passage through *Stellaria media* probably does not play an economic role, since this plant is common only in moist, shady locations, unfavorable to the curly top vector, *Eutettix tenellus* (Baker). If, however, sugar beets in a very susceptible stage or some of the important weed hosts of the beet leafhopper known to be susceptible to curly top were found to act in a manner similar to that of *S. media* in restoration of virulence to attenuated virus, the findings might have a bearing on the curly top situation. Work was undertaken on sugar beets in the seedling stage and on alfileria, *Erodium cicutarium* (L.) L'Hér., a host plant occurring in considerable abundance in many leafhopper breeding areas. Later, peppergrass, *Lepidium nitidum* Nutt., which is also an important host, was tested. These plants were known to be highly susceptible to virulent curly top virus. In the case of alfileria, it is known that the curly top virus can be carried through the winter on this species and it may be a winter and early-spring host plant in California for the beet leafhopper. Severin [6] includes peppergrass as one of the important overwintering annuals for this insect.

MATERIALS AND METHODS

The experimental procedure used in the tests has been described.[7] The virulent curly top virus was first attenuated by passage through *Chenopodium murale*. This attenuated virus was then inoculated by means of leafhoppers into the susceptible plant, after which the viru-

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[2] Acknowledgment is made to Eubanks Carsner and N. J. Giddings, senior pathologists, and G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, for their criticisms in the preparation of this manuscript.

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lence of the virus was determined by transmission to young, fast-growing sugar beets in the two- to four-leaf stage. As a control in each test, inoculations were made with the virulent virus before and after its passage through *C. murale*.

In the first experiments with alfileria, the type of symptoms was described as mild or severe as in the work reported for *Stellaria media*.[7]

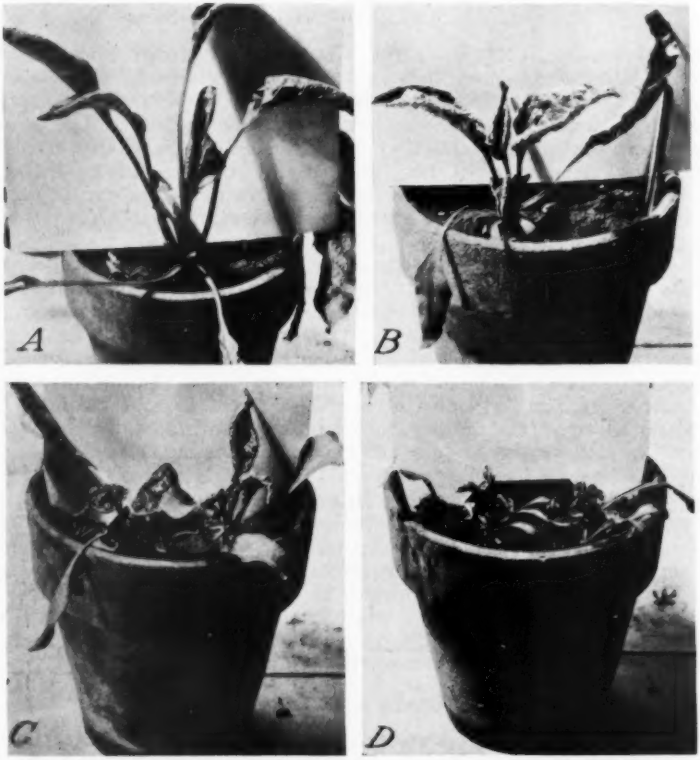

FIGURE 1.—Curly top-infected beets showing range of severity of symptoms: *A*, Mild, grade 2; *B*, intermediate, grade 3; *C*, more severe, grade 4; and *D*, extreme dwarfing, grade 5. (Grade 1, mild response characterized by veinlet clearing only, not illustrated.)

In later experiments a grading system to express severity of the symptoms on test plants as developed by Giddings [8] was adopted. The affected plants were classified in five grades based on response, as follows:

(1) *Mild response.*—No stunting or dwarfing; veinlet clearing or slight vein enlargement only visible symptoms.

(2) *Mild response.*—Little or no dwarfing; veinlets cleared, vein roughening and vein protuberances on some leaves, and leaves sometimes curled (fig. 1, *A*).

(3) *Intermediate response.*—Some dwarfing and stunting, practically all leaves involved; leaves somewhat curled, vein roughening on nearly all leaves (fig. 1, *B*).

[7] LACKEY, C. F. See footnote 5.

[8] GIDDINGS, N. J. A GREENHOUSE METHOD FOR TESTING RESISTANCE TO CURLY TOP IN SUGAR BEETS. Phytopathology 27: 773-779, illus. 1937.

(4) *More severe reaction.*—Dwarfing severe, leaves markedly curly or distorted, veins conspicuously distorted and roughened (fig. 1, *C*).

(5) *Extreme dwarfing.*—All symptoms of preceding grade very pronounced; plants nearly killed (fig. 1, *D*).

EXPERIMENTAL TESTS WITH SUGAR BEETS

Sugar beets (*Beta vulgaris* L.) in the cotyledon stage, the two- to four-leaf stage, and the six- to eight-leaf stage were tested for their effect on the attenuated virus from *Chenopodium murale*. The cotyledon beets showed 63.8 percent infection with an average severity of

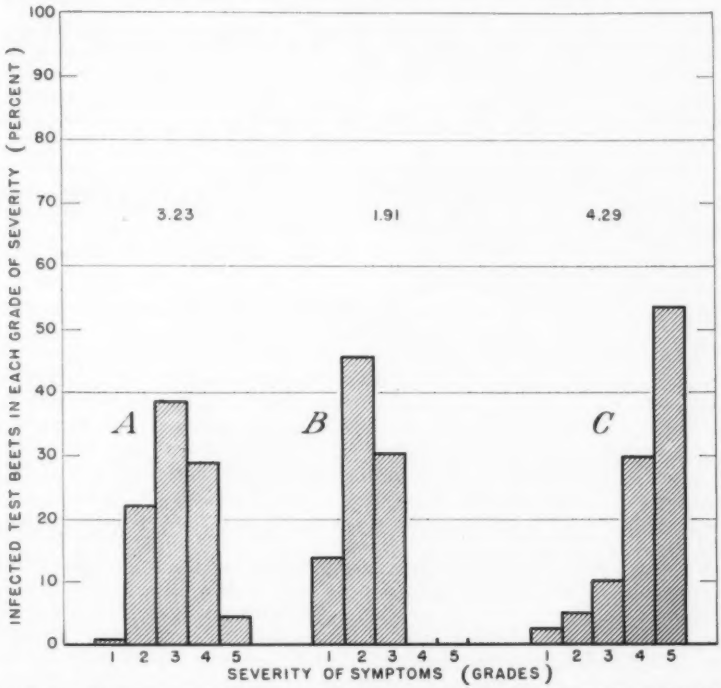

FIGURE 2.—Histograms showing distribution of symptoms in grades of severity in test beets infected with attenuated curly top virus before and after passage through cotyledon-size sugar beets: *A*, Attenuated virus after passage through cotyledon beets; *B*, attenuated virus untreated; *C*, original virulent virus. (Numbers above histograms indicate average grade of severity of symptoms for each treatment.)

2.31; two- to four-leaf beets, 42.2 percent infection with an average severity of 1.91; and the six- to eight-leaf beets, 34.0 percent infection with an average severity of 1.34. Twenty-three tests in which two- to four-leaf beets were inoculated with the original virulent virus gave an average grade of severity of 4.29 (fig. 2). Table 1 is a summary of the results of 2 years' trials with these various-sized beets.

The cotyledon beets showing symptoms severe enough to grade 4 and 5, comprising 13.4 of those infected, were tested for their effect on the attenuated virus. It is evident (table 2) that some degree of restoration was obtained by passage through cotyledon beets. In the course of these tests with sugar beets, two cases occurred in which

some increase of virulence resulted from passage of the virus through sugar beets in the two-leaf stage. Beets with six to eight leaves did not increase the virulence of the attenuated virus. Table 2 summarizes the results of these experiments, which are presented in more detail in the histograms in figure 2.

As an additional measure of the effect of passing the attenuated virus through cotyledon beets, the infected test beets in several experiments were weighed 8 to 10 weeks after inoculation. The beets inoculated with the attenuated virus averaged 15.16 g, green weight; while those inoculated with the restored virus averaged 7.87 g.

TABLE 1.—*Summary of all experiments in which sugar beets in the cotyledon stage and those in the 2- to 8-leaf stages were inoculated with the attenuated virus*

Size of beets when inoculated	Beets inoculated	Beets infected	Average grade of severity	Infected beets in each grade of severity [1]				
				1	2	3	4	5
	Number	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Cotyledon stage	304	63.8	2.31	18.0	46.9	21.1	10.8	2.6
2- to 4-leaf stage	95	42.2	1.91	38.0	40.5	4.7	9.5	0
6- to 8-leaf stage	103	34.0	1.34	68.6	31.4	2.9	0	0

[1] Percentages based on number of infected beets.

TABLE 2.—*Restoration of virulence of attenuated virus by passage through cotyledon beets*

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
Passed through cotyledon beets	32	500	73	3.23±0.11
Not passed through cotyledon beets	25	235	59	1.91± .10

EXPERIMENTAL TESTS WITH ERODIUM CICUTARIUM (ALFILERIA)

Evidence from the first experiments indicating that passage of the attenuated virus through alfileria may restore the virulence is shown in table 3. All cases in which some degree of restoration was obtained are included in this résumé. From the data given in table 3, it is clear that after a single passage attenuated virus may be so restored in virulence as to produce severe symptoms on the test beets. These results are comparable to those reported for *Stellaria media* [9] in that there were many cases in which alfileria did not restore the virulence of the attenuated virus.

TABLE 3.—*Results of passing the attenuated and virulent forms of curly top virus through alfileria*

Treatment and form of virus used	Beets inoculated [1]	Beets infected	Type of symptoms
Not passed through alfileria:	*Number*	*Percent*	
Virulent	5	60	Severe.
Attenuated	25	8	Very mild.
Passed through alfileria:			
Virulent	40	60	Severe.
Attenuated	52	52	Do.

[1] 3 to 6 leafhoppers were fed on each test beet for 3 to 4 days.

[9] LACKEY, C. F. See footnote 5.

The results of later and more extensive experiments are summarized in table 4. Only 50 percent of the alfileria plants inoculated with attenuated virus showed symptoms of curly top. About 90 percent of these diseased plants were tested for their effect on the attenuated virus. Of these, only 20 percent increased the virulence of the virus to some degree. Before passage through alfileria, the attenuated virus produced only 32 percent infection on the test beets, with an average grade of severity of 1.82, as compared with 63 percent infection, with an average grade of 3.23, after passage through alfileria. This demonstrates that alfileria occasionally increases the virulence of the attenuated virus.

TABLE 4.—*Restoration of virulence of attenuated virus by passage through alfileria*

[Summary of experiments conducted at Riverside, Calif., 1931–34]

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
After passage through alfileria	55	1,574	63	3.23±0.09
Before passage through alfileria	45	840	32	1.82± .06

In 17 of these experiments test beets were inoculated with the original virulent virus. The average grade of severity of symptoms produced by this virus was 4.22 (fig. 3).

The histograms in figure 3 show what percentage of the infected test beets fell into each grade of severity. The test beets infected with the attenuated virus after passage through alfileria showed symptoms that placed a majority of them in grades 3 and 4 and a few in grade 5. Figures 4 and 5 show this variation in degree of severity of symptoms expressed by the attenuated virus after restoration. On the other hand, the attenuated virus before treatment produced symptoms which in the greatest number of cases graded 1 and 2. While most of the test beets inoculated with the original virulent virus showed symptoms grading 4 and 5, some of these plants exhibited symptoms mild enough to put them in grade 1, 2, or 3. As shown in studies on restoration through *Stellaria media*,[10] the appearance of mild symptoms on some of the test beets that had been inoculated with the restored virus or with the original virulent virus appears to be an individual plant response in some instances. An apparently comparable situation sometimes develops in the case of alfileria, as illustrated in figure 5. Transferring virus from such mildly affected plants to test beets resulted in severe symptoms in the majority of cases.

EXPERIMENTAL TESTS WITH LEPIDIUM NITIDUM (PEPPERGRASS)

The attenuated virus used in the test with *Lepidium nitidum* was from two sources. The first lot had been attenuated by passage through *Chenopodium murale*, as in all the previously mentioned experiments on restoration. The second lot was obtained from leafhoppers collected in the field from natural breeding areas. Only 40 percent of the *L. nitidum* plants inoculated with the attenuated virus became diseased, and 70 percent of these infected plants increased the virulence of the virus. The histograms in figure 6 show the results of these tests. The attenuated virus harbored by field collections

[10] LACKEY, C. F. See footnote 5.

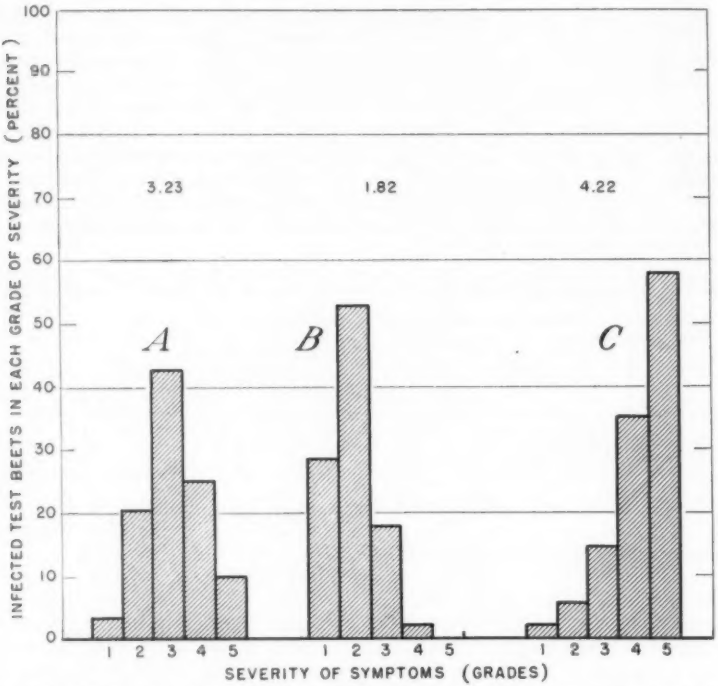

FIGURE 3.—Histograms showing distribution of symptoms in the different grades of severity on test beets infected with the attenuated virus before and after passage through alfileria: *A*, Attenuated virus after passage through alfileria; *B*, attenuated virus untreated; *C*, original virulent virus. (Numbers above histograms indicate the average grade of all test beets for the different treatments.)

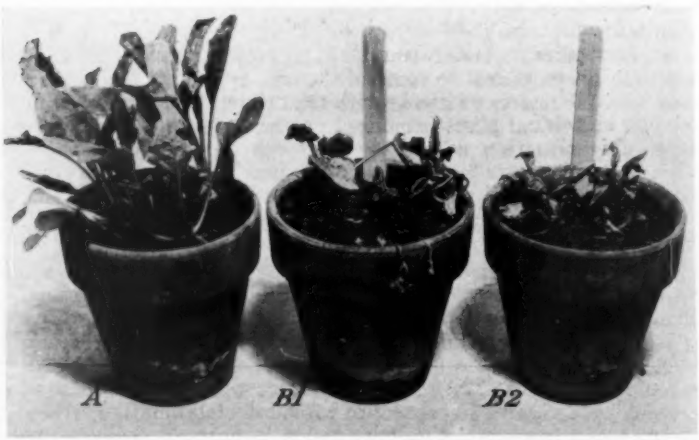

FIGURE 4.—Symptoms produced on test beets by restored and by untreated attenuated virus: Pot *A*, by attenuated virus before passage through alfileria; pots *B*1 and *B*2, by the same virus after passage through alfileria.

FIGURE 5.—Symptoms produced by the restored and the untreated attenuated virus on test beets: Pot *A*, by attenuated virus before passage through alfileria; pot *B*, by the same virus after passage through alfileria, showing variation in individual plant response to infection.

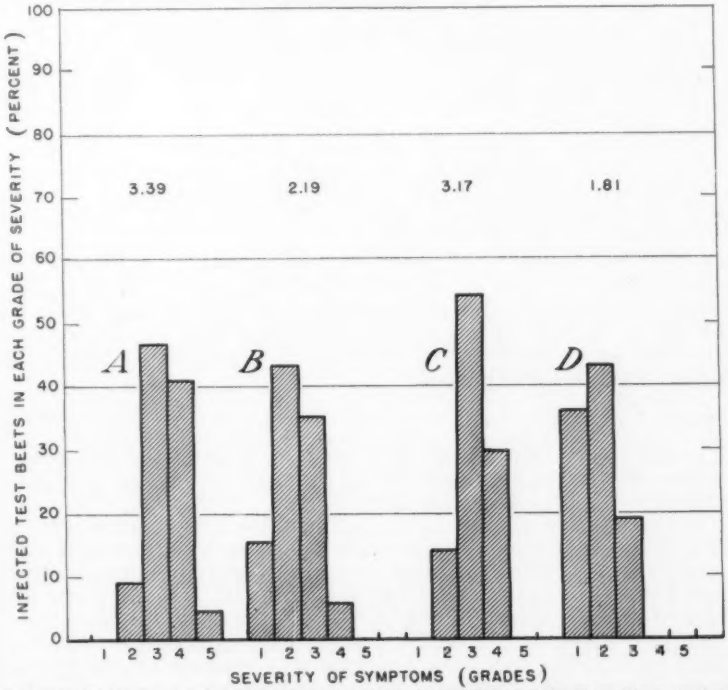

FIGURE 6.—Histograms showing distribution of symptoms in the different grades of severity on test beets infected with the attenuated virus before and after passage through *Lepidium nitidum: A*, Attenuated virus from field collection of insects, passed through *Lepidium nitidum; B*, attenuated virus, from field collection, untreated; *C*, attenuated virus, from *Chenopodium murale*, passed through *Lepidium nitidum; D*, attenuated virus, from *C. murale*, untreated. (Numbers above histograms indicate average grades of severity.)

of leafhoppers, with an average grade of 2.19, was not attenuated so much as the virus that passed through *C. murale*, with an average grade of 1.81. It would appear at first glance at the histograms that the attenuated virus from the field leafhoppers was restored to a greater degree, but it showed an increase in the average grade of severity of only 1.20 grade points while the attenuated virus from *C. murale* showed an increase of 1.36 grade points. The reaction to passage through peppergrass by the attenuated virus from the two sources is therefore very similar. Table 5 summarizes all the tests made with these two lots of attenuated virus. The average grade of severity before and after treatment is practically the same as that in the restoration tests with alfileria.

TABLE 5.—*Restoration of virulence of attenuated virus by passage through Lepidium nitidum*

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
After passage through *Lepidium nitidum*	13	135	84.51	3.26±0.27
Before passage through *Lepidium nitidum*	11	85	69.41	2.10± .50

DISCUSSION

The economic bearing of the studies with wild hosts on the curly top problem will require further investigation in the field. However, the experimental evidence with young sugar beets suggests that an increase in the virulence of the virus may occur under field conditions also.

Observations on alfileria indicated that virulence restoration occurred most frequently in plants that were growing rapidly. To test the correctness of this idea, plants of different ages were fertilized with nitrogen (ammonium sulphate) 10 days before inoculation. When very young alfileria plants or those given extra nitrogen were used, a greater number became severely diseased and restored the virus. Similarly, inoculation with eight leafhoppers resulted in more cases of severe infection and consequently more plants producing restoration than when inoculations were made with two leafhoppers. None of these various procedures had any pronounced effect on the degree of restoration attained.

SUMMARY

Virulent curly top virus, attenuated by passage through *Chenopodium murale*, has been occasionally restored in virulence by passage through cotyledon-sized sugar beets.

The appearance of mild symptoms on a few test beets inoculated with restored virus or with the original virulent virus seems in some cases to be an individual plant response, since virus from some of these beets produces severe symptoms on the majority of young test beets to which it is transferred.

Alfileria (*Erodium cicutarium*) and peppergrass (*Lepidium nitidum*) also occasionally restore the virulence of the attenuated curly top virus. These plants are important overwintering hosts for both the leafhopper vector and the virus.

The probable economic aspect of these investigations is suggested.

BIOLOGICAL VALUE OF CASEIN AS A SUPPLEMENT TO THE PROTEINS OF BARLEY IN RATIONS FOR PIGS[1]

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INTRODUCTION

It has been shown at the California Agricultural Experiment Station by Thompson and Voorhies (*7*)[2] and by Hughes (*2*) that barley, when fed as the only source of protein, does not produce optimum growth in pigs. Protein-rich concentrates like fish meal and tankage or dairy byproducts when used as supplements to barley, produce more rapid growth. Such concentrates, however, contain other nutrients than protein, which makes it unsafe to conclude that the protein is responsible for the results obtained. Casein, since it has been used by biologists in similar studies with smaller animals than the pig, and since it is relatively low in cost, was used as a supplement in the studies reported here to determine whether or not the proteins of barley are present in amounts necessary for the rapid development of the pig.

EXPERIMENTAL DATA

In June 1929 two pigs about 50 pounds in weight, having free access to direct sunlight, were put on a diet of rolled barley, salt, and cod-liver oil. They grew very slowly and became stiff in the joints. Early in January of the following year calcium carbonate was added to the diet. At 1 year of age the pigs weighed only 162 pounds. It was believed that the diet was sufficient in all known factors for some growth, but the pigs had not gained in weight for some time. The only protein in the ration was that of barley. Casein was added to the diet and an immediate response was manifested by increase in weight, thrift, and appetite. During the following 3 months the pigs made an average daily gain of slightly more than 1 pound and became normal in health.

The results of this experiment were significant, but the number of animals used was too small to make possible any definite conclusions; therefore, a similar experiment on a larger scale was conducted in 1931–32. Two groups of eight pigs each were divided as nearly as possible into comparable lots. Each group was placed in a concrete lot with free access to direct sunlight, and the pigs were fed all they would consume of the following diets:

Group 1. Rolled barley, 98 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and 5 cc of cod-liver oil each daily (basal diet).

Group 2. Rolled barley, 96.5 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; casein, 1.5 percent; and 5 cc of cod-liver oil each daily.

The results of this experiment are summarized in table 1.

[1] Received for publication Mar. 9, 1937; issued October 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 465.

The average beginning and final weights were, for group 1, 23.3 and 94.6 pounds; and for group 2, 25.0 and 203.8 pounds. During the first part of the experiment the pigs fed casein (group 2) gained three times as fast and were more economical in their use of food than were the pigs fed protein supplied only by the barley (group 1). Each pound of casein fed to the animals in group 2 had a replacement value of 49.5 pounds of the basal feed. When casein was added to the diet of group 1, at both the 1.5- and 5-percent levels, there was an immediate response in increased appetite, rate of gain, and economy of feed utilization. The pigs did not gain as rapidly at either level as the pigs in group 2; however, they did consume less food for a unit of increase. It is postulated that this was due to a lower maintenance requirement because of a marked difference in the size of the pigs in both lots. When the casein level was increased to 5 percent the animals in both groups gained more rapidly and consumed less food for 100 pounds of gain than at any other time during the progress of the experiment, the protein level being apparently more nearly optimum for pigs at this stage of growth and development. The rate of gain in both groups was just about twice as rapid as when casein was fed at the 1.5-percent level.

TABLE 1.—*Summary of data from the second and third experiments to show the effect of adding casein to the basal diet of pigs when the sole other source of protein was barley*

SECOND EXPERIMENT [1]

Feeding period and diet	Group 1		Group 2	
	Average daily gain	Feed consumed per 100 pounds gain in weight	Average daily gain	Feed consumed per 100 pounds gain in weight
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 23 to Dec. 12, 1931 (group 1, basal diet: group 2, 1.5 percent casein added)	0.16	886.7	0.50	508.9
Dec. 12, 1931, to Jan. 23, 1932 (both groups, basal diet plus 1.5 percent casein)	.39	493.9	.77	529.9
Jan. 23 to Feb. 13, 1932 (both groups, basal diet plus 5 percent casein)	.88	310.0	1.45	339.3
Feb. 13 to Mar. 5, 1932 (both groups fed basal diet only)	.14	2,265.0	.74	711.3

THIRD EXPERIMENT [2]

May 12 to Oct. 6, 1934 (group 1, basal diet; group 2, 1.5 percent casein added)	.36	577.6	.62	431.8
Oct. 6, 1934, to Jan. 10, 1935 (both groups, basal diet plus 1.5 percent casein)	1.20	[3] 445.1	1.37	438.9

[1] Groups of 8 pigs each.
[2] Groups of 10 pigs each.
[3] One pig was "off feed" for some time and died the day the experiment closed.

While the last period was only a short one, the results are significant because of the immediate decrease in daily gain by both groups and the large increase in food consumption required for a unit of increase in weight. The pigs in group 1 reverted to their original rate of gain, and while the pigs in group 2 were much larger and were fattening, their rate of increase dropped from 1.45 to 0.74 pounds per head daily.

A third experiment was conducted in 1934 to further check the results already obtained. This experiment was begun May 12, 1934,

and concluded January 10, 1935. Two groups of 10 pigs each were fed diets similar to those previously used. The average beginning weight for both groups was 29.6 pounds. The conditions of the experiment, including the grouping of the pigs and the size of the pens, were the same as before. To be sure that a lack of vitamin A would not influence the results, each pig in both groups was given 5 cc of cod-liver oil daily until July 21, and from that time until the experiment was concluded each was given 10 cc. The results are presented in table 1.

In this experiment the pigs were larger and thriftier at the beginning than those of the second experiment and were not fed so long in the first period, which may in part account for the difference in the rate of gain and the replacement value of the protein when the results are compared with those of the second experiment. Each pound of casein fed group 2 had a replacement value of 22.5 pounds in terms of food saved over that fed group 1. During the second phase of this experiment the pigs in both groups gained more rapidly than in the first period, which is in agreement with the results of the other experiments. While in some details the results of this test differed slightly from those of the preceding one, in principle they were the same.

In 1936 a fourth experiment was concluded which differed somewhat from those already reported. Osborne and Mendel (*3*) had reasoned that the failure of cereal proteins as they are generally fed is due to a lack of sufficient total cereal protein. In an effort to prove or disprove this theory and to obtain further information on the original subject, barley proteins fed group 1 were increased to a point in excess of that fed group 2, which received barley and casein. This was done by adding dried brewers' grains from which some of the hulls had been removed (the material removed was a mixture of hulls and some grain). Since the addition of dried brewers' grains to the diet of group 1 increased the fiber content, barley hulls were added to the mixture fed group 2 to make it comparable in this respect. The total crude protein fed groups 1 and 2 was respectively 11.12 and 10.42 percent. The fiber content of the ration fed to group 1 was 1 percent higher than that of the ration fed group 2. It is probable, however, that there was little difference in the amount of fiber consumed by the pigs in the two groups since those of group 1 left considerable quantities of hulls in the bottom of their feeder.

Since commercial casein contains lactoflavin, and the results of other experiments with hogs at this station (unpublished data had indicated that this factor is necessary for normal growth, it was deemed expedient to feed a third group of pigs casein washed free of this factor. The casein was washed by the method of Evans, Lepkovsky, and Murphy (*1*), modified by that of Supplee, Flanigan, Hanford, and Ansbacher (*6*).

The crude protein and fiber content of the diets fed groups 2 and 3 were the same.

The diets were:

Group 1. Rolled barley, 88 percent; brewers' grains (dried), 10 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and 10 cc of cod-liver oil each weekly.

Group 2. Rolled barley, 91 percent; barley hulls, 5 percent; casein, 1.5 percent; sodium chloride, 1 percent; calcium carbonate, 1.5 percent; and 10 cc of cod-liver oil each weekly.

Group 3. The same as for group 2 except that the casein fed was washed free of lactoflavin.

The physical conditions of this experiment, including the grouping of the pigs and the size of the pens, were comparable to those of the other experiments. The experiment was begun June 6 and concluded October 31, 1936. There were eight pigs in groups 1 and 2 and five pigs in group 3.

TABLE 2.—*Summary of data from the fourth experiment to show the effect of adding commercial casein and casein washed free of lactoflavin to the basal diet of pigs when the sole other source of protein was barley*

[Experiment begun June 6 and concluded Oct. 31, 1936; 8 pigs in groups 1 and 2 and 5 in group 3]

Group no.	Average initial weight	Average daily gain	Feed consumed per 100 pounds gain in weight
	Pounds	*Pounds*	*Pounds*
1	39.3	0.61	383.0
2	38.8	1.12	320.4
3	34.4	.57	423.7

In this experiment as in the others the pigs fed casein (group 2) gained much faster and consumed less feed for 100 pounds of gain than those (group 1) fed only barley proteins, even though the total protein in the diet of group 1 was greater than that of group 2. In this case each pound of casein in the diet of group 2 had a replacement value of about 13 pounds of the diet fed group 1. However, if the results of this experiment are compared with those for animals fed only barley as shown in table 1, it will be found that the additional protein supplied by brewers' grain did result in increasing the daily gains and in decreasing the amount of feed required for 100 pounds of gain.

The pigs in group 3 (fed casein washed free of lactoflavin) gained no faster than the pigs of group 1 and consumed more feed for a unit of increase than either of the other groups.

DISCUSSION

In the experiments reported herein where the proteins of barley were the only source of protein for young growing pigs the rate of growth was slow. This agrees with the finding of Steenbock, Kent, and Gross (*5*) and with that of McCollum, Simmonds, and Parsons (*3*). Osborne and Mendel (*4*), however, reported that barley proteins are adequate as a whole in the nutrition of growth, and that several of their rats grew to large adult size without any other source of protein. The growth increase in their rats, however, was not consistent.

The addition of casein to the diet in the present experiments reduced considerably the amount of feed required for a unit increase in weight. Its value in replacing barley varied; however, 1 pound of casein supplanted more than 20 pounds of barley, except in the fourth experiment when it replaced about 13 pounds. In this case barley proteins were increased by the addition of dried brewers' grains to a point above that of the other group fed barley and casein. The results of these studies indicate that barley is deficient in some essential factor or factors necessary for normal growth and weight increase in the young pig.

That the total amount of protein in barley is probably a factor of importance is indicated by the fact that when dried brewers' grains were added to barley, thereby increasing the total barley proteins fed, the gains made were greater and the amounts of feed consumed for a unit of gain were smaller than when barley was fed alone.

Some uncertainty exists as to the reason for the beneficial effect of the addition of casein. The quantity of the protein and the lactoflavin content of the casein have been considered. Pigs fed unwashed commercial casein (group 2, experiment 4) gained twice as fast as pigs fed the same diet at the same time except that casein (from the same sack) washed free of lactoflavin was used.

SUMMARY

Young growing pigs fed a diet in which the proteins of barley were the only source of protein grew very slowly and required large quantities of feed for a unit of increase in weight.

When casein was added to such a diet there resulted a marked increase in rate of growth and a large reduction in feed required for an increase in body substance.

When the barley proteins in the diet were increased by the addition of dried brewers' grains, the gains were more rapid and the feed consumed for 100 pounds of gain were less than when only barley was fed.

The possibility that the lactoflavin in casein is responsible for its beneficial effect when added to barley is suggested.

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EFFECT OF PROTEIN DEFICIENCY IN THE RATION ON THE AMOUNT OF FEED CONSUMED BY LAMBS[1]

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INTRODUCTION

In studies of the nutritive value of proteins in which biological values are to be calculated (*3*),[3] it is necessary to know the losses of endogenous and metabolic nitrogen. To measure such losses, the experimental animals are fed diets which contain no nitrogen, or very little. Since diets of this type are not only deficient in protein, but are radically different from ordinary diets, certain difficulties have been experienced in using them.

A serious limitation in the use of most of the nitrogen-free or low-nitrogen diets has been the difficulty of obtaining adequate feed consumption. With the possible exception of swine, it has been found almost impossible to induce experimental animals to consume satisfactory amounts of these diets for the desired length of time. In most cases when such diets were readily consumed, they contained a certain amount of some high-protein feed which was added specifically to increase the palatability.

A palatable ration, free from nitrogen or very low in nitrogen, has been particularly difficult to devise for lambs. An attempt was made at this station (*7*) to feed lambs a purified diet almost free from nitrogen and containing no natural roughage, but the ration was not sufficiently palatable to the lambs and its use was discontinued. Workers who have used low-nitrogen rations for lambs (*4*, *5*, *6*, *7*, *8*) have included straw as part of the ration, but even when this was done the feed intake on the low-nitrogen rations was decidedly smaller than on the experimental rations containing adequate protein, and in practically all cases the lambs lost in weight.

The object of the present study was to devise a low-nitrogen ration, containing only purified ingredients, which would be consumed readily by lambs over a reasonable length of time. Such a ration was desired for use in experiments conducted at the Cornell Station on the nutritive value of proteins.

PRELIMINARY TRIALS

Considerable work has been done at this station in developing purified or synthetic diets for Herbivora (*2*, *9*). A diet for sheep which has given good results over a long period of feeding is as follows: Regenerated cellulose,[4] 20 percent; casein, 20 percent; sucrose, 15 percent; starch, 28 percent; yeast, 7 percent; salt mixture,[5] 4 percent;

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[2] The author acknowledges his indebtedness to P. E. Newman and J. E. Foster of this station for assistance in the conduct of part of the work. Grateful appreciation is also extended to Dr. C. M. McCay, of the animal nutrition Laboratory, for helpful suggestions relative to this study.

[3] Reference is made by number (italic) to Literature Cited, p. 473.

[4] This product was obtained as washed Sylphrap from the Sylvania Industrial Corporation, New York City.

[5] The salt mixture used was one designed by Woodward and McCay (*9*) for use in synthetic diets for Herbivora.

lard, 6 percent; and a vitamin A–D supplement. This diet, as well as other very similar diets, is apparently palatable to sheep and has been consumed in such amounts that good growth has resulted. However, it should be noted that this mixture contains a high percentage of protein.

With this work as a basis, the most logical procedure to follow in developing a low-nitrogen diet was apparently to replace the casein with starch. This change would not be expected to affect the palatability of the mixture very radically. That some difficulty might be encountered, however, became apparent, after some preliminary work which was made in connection with another problem.

In the preliminary study, a growing lamb which previously had been getting a common, practical ration was fed a diet containing 18 percent of casein but otherwise similar to the purified diet described above. No trouble was experienced in getting satisfactory feed consumption, and so it was concluded that this 18-percent casein diet was palatable to the lamb. Then, by using a larger percentage of starch and a smaller percentage of casein, another diet was prepared in which the casein made up only 8 percent of the total ration. When first fed this mixture seemed just as palatable as the 18-percent casein diet and the same amount of feed was consumed. At the end of 6 days, however, the lamb went off feed and was emaciated in appearance. At about the same time the feces became loose in character and offensive in odor, indicating acute indigestion. Although the feeding of this diet was continued for 12 days longer, the lamb made little or no improvement and the former level of feed consumption was not regained.

The lamb was then fed a similar diet except that it contained 14 percent of casein. After a short time the lamb's appetite became stronger and more regular, the feces were more normal, and the lamb was apparently in considerably better physical condition.

From these results it seemed unlikely that omitting all the casein from the diet and adding a corresponding amount of starch would make a satisfactory low-nitrogen diet for lambs. Therefore, certain other modifications were made to the original purified diet in addition to the substitution of starch for casein. This mixture, which was used in all the remaining studies on low-nitrogen diets, is given in table 1. Another diet, similar in all respects except that 20 percent of casein replaced a corresponding amount of starch, was also used. This diet, hereafter referred to as the "casein diet", is also given in table 1.

TABLE 1.—*Composition of experimental diets*

Ingredients	Low-nitrogen diet	Casein diet	Ingredients	Low-nitrogen diet	Casein diet
	Percent	*Percent*		*Percent*	*Percent*
Regenerated cellulose	18.0	18.0	Yeast	5.0	5.0
Casein		20.0	Minerals [1]	4.5	4.5
Starch	53.5	33.5	Anise oil	[2] T	[2] T
Brown sugar	15.0	15.0			
Corn oil	4.0	4.0	Total	100.0	100.0

[1] The 4.5 percent of minerals was made up 2.5 percent of the Hawk and Oser modification of the Osborne and Mendel mineral mixture (*1*) and 2.0 percent of a mixture of ground limestone 40 parts, steamed bonemeal 40 parts, and salt 20 parts.

[2] T = trace.

As may be noted in table 1, brown sugar was used instead of cane sugar, and corn oil was added instead of lard. This was done in an effort to increase the palatability of the diet. A different mineral mixture also was used. To these diets, a small quantity of anise oil was added. This was done to impart a pleasant and distinct taste, as well as odor, to the diet, which was otherwise deficient in these respects. It was hoped that the addition of this material would aid in securing greater feed consumption. Anise oil can be added readily to any ration, and by using it abrupt changes may be made from one ration to another without very much danger of the lambs refusing the new ration because of the difference in taste or smell.

In order to correct for any possible decrease in the palatability of the mixture that might result from the presence of such a large amount of fine starch, the starch was cooked together with the other constituents of the diet, except the yeast and minerals. This cooked material was thoroughly dried in a drying room and then coarsely ground. Later the minerals and yeast were added to complete the diet.

The diet as fed was a brownish, rather gritty material, somewhat like small, hard seeds. It was sweet to the taste and had a noticeable odor, as well as taste, of the anise oil. From the standpoint of taste, odor, and physical condition, the diet seemed about as palatable as could be devised with the ingredients used. However, no direct test was made to determine whether these modifications actually did improve the palatability of the mixture.

EXPERIMENT 1

For the first experiment a thrifty grade lamb was obtained from the university flock. The lamb was fed a ration of ground corn, linseed meal, and timothy hay for a short time, and then the low-nitrogen diet was gradually substituted for part of the concentrates until the lamb received only the low-nitrogen diet plus the hay. So far as could be determined, the low-nitrogen diet was just as readily consumed as the concentrate mixture. After this substitution, the allowance of the purified diet was increased and the amount of hay was decreased. Within a few days the lamb was receiving only the low-nitrogen diet.

The low-nitrogen diet was eaten readily and remained very palatable for the first 2 days that it was given as the only feed. On the third day, however, the lamb refused some feed and later went badly off feed. The feed consumption was decidedly under the former level, and the lamb became irregular in its eating. The feces were loose and had a bad odor. Without question, the appetite and the digestive processes of the lamb were badly upset after this short period on the low-nitrogen diet.

The question naturally arose as to why the feed, which at first had apparently been eaten with relish, should suddenly become objectionable to the lamb. It was thought that possibly the change to the purified diet had been too abrupt. However, there were no definite indications that such was the case. The most logical explanation appeared to be that the low-nitrogen diet had seriously disturbed the digestive system of the lamb and so had greatly reduced its desire for food.

After a few days, the feeding of the low-nitrogen diet was discontinued and the lamb was again fed the ordinary barn feeds. Although

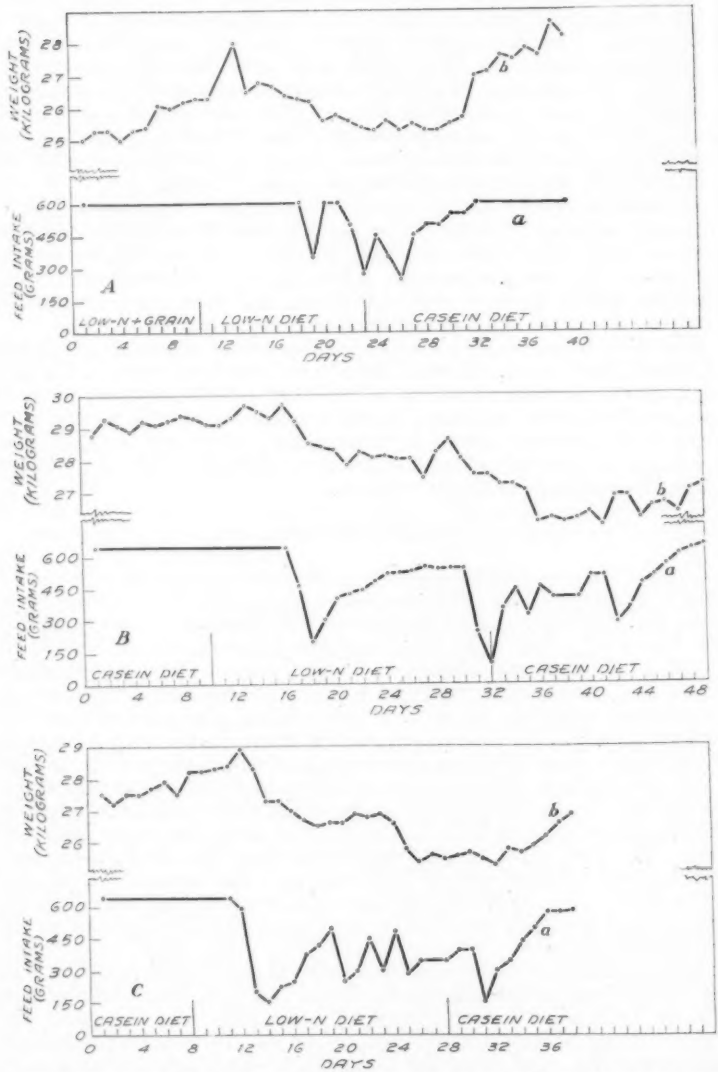

FIGURE 1.—Feed intake (*a*) and body weight (*b*) of lambs 1 (*A*), 2 (*B*), and 3 (*C*) while on the indicated experimental diets.

its improvement was slow in the beginning, by the end of 10 days it was again eating satisfactorily and gaining in weight. Evidently it had completely recovered from the period of feeding on the low-nitrogen diet.

It was then decided to check these results by repeating the procedure with the same lamb. This time, however, instead of decreasing the amount of grain as in the first trial, the amount of hay was decreased as the low-nitrogen diet was added. After a few days, the hay was entirely removed so that the lamb was getting about one-third of the relatively high-protein concentrate mixture (corn and linseed meal) and two-thirds of the low-nitrogen diet. The lamb readily consumed this mixture and made considerable gain in weight. After a period of 10 days, it was fed only the low-nitrogen diet. The effect of this change in diet on the feed consumption and on the daily body weights throughout the experiment are shown in figure 1, *A*.

As figure 1, *A*, *a*, indicates, at the beginning the lamb ate the same amount of the low-nitrogen diet that it had of the diet plus the corn and linseed-meal mixture. Here, again, was evidence that the low-nitrogen diet was at first just as palatable as the natural feeds. At the end of 8 days, however, the lamb refused to clean up its feed. It had the appearance of being in extreme discomfort and was not so active as formerly. The feces lost their typical pellet shape and gave off a strong odor. In practically all respects the lamb had developed the same condition as when previously fed this same diet.

Instead of bringing the lamb back to a normal condition by giving the natural feeds, it was fed a purified diet containing considerable casein. This diet was prepared exactly as the low-nitrogen diet had been except that in this mixture casein was added to the extent of 20 percent of the total diet and the percentage of starch was reduced accordingly. By reason of the cooking and the addition of anise oil, the odor, taste, and physical condition were the same. Even the color was nearly the same, the casein diet being just a trifle darker. No particular difference in the desire for feed was observed when the casein diet was first offered to the lamb, but after a few days, it developed a stronger and more uniform appetite, and at the end of 8 days, it was eating the former amount of feed. The casein diet was fed for 8 additional days, during which time the lamb became normal in all respects and had an excellent appetite for the diet.

The changes in the body weight of the lamb during the experiment are of interest. As shown in figure 1, *A*, *b*, the lamb made unusually large gains during the period in which the concentrate mixture was fed along with the low-nitrogen diet. The extent of these gains was no doubt due in part to the previous emaciated condition of the lamb. The lamb also continued to gain for a few days on the low-nitrogen ration, but after a rather marked increase in weight, it began to lose rapidly. This loss of weight corresponded to the period in which it had diarrhea. Shortly after changing to the casein diet, the lamb improved in body weight, and by the end of the experiment it was making good gains.

EXPERIMENT 2

Experiment 2 was conducted primarily as a check upon the first experiment. While the low-nitrogen diet had proved unsuccessful in two attempts with the same lamb, it was believed desirable to try the

same diet with additional lambs before admitting failure. Also, the behavior of the first lamb on each of the diets had been of especial interest and further observations seemed desirable. All of the rations used in this experiment were the same as those used in experiment 1.

Two grade lambs were secured for this study. They were very similar to the lamb used in the first experiment, but were somewhat heavier in weight. The 20-percent casein diet was gradually substituted for the customary barn ration, and after the lambs had been on the casein diet for a period of time, they were changed to the low-nitrogen diet. The casein diet was fed again at the end of the experiment. The daily feed intake and the daily weights throughout the period are shown in figure 1, *B* and *C*.

The results secured with lambs 2 and 3 were similar to those obtained with lamb 1. The lambs apparently did not notice when the change was made from the casein diet to the low-nitrogen diet. Their feed intake remained the same for a few days and their appetites were good. Then suddenly they began to refuse feed, and for the duration of the period on the low-nitrogen diet, they would not consume the feed at the former level of intake. The condition of their feces was also abnormal.

A change was made back to the casein-containing ration, and, again, insofar as could be determined, the lambs did not observe the change in their feed. After a few days, however, a noticeable improvement was made and both lambs almost completely regained their former level of feed intake and appeared to be in good condition. Considerable increases in body weight were also made.

DISCUSSION

The data presented bring out clearly the fact that there are important factors in rations other than physical appearance, taste, or smell which may determine the amount of a feed or a ration that an animal will consume. Insofar as could be judged by inspection, the casein diet and the low-nitrogen diet were practically identical. The cooking and subsequent grinding of most of the constituents in each diet made them markedly similar in appearance and the taste and odor were almost identical, owing primarily to the addition of the anise oil. The accuracy of these observations is borne out by the fact that in no instance when the diets were changed did the lambs give any indication that they noticed the change. It therefore seems logical to conclude that both diets were palatable and that the difference in feed consumption was due to the presence of the casein or more protein in one of the rations.

The low-nitrogen diet as prepared did contain an appreciable amount of protein furnished by the yeast. While this amount of protein would not have been sufficient to maintain the lambs adequately over a long period, it seems unlikely that the lack of even this essential nutrient would become apparent in so short a time. Probably the poor results obtained on this diet were due to the severe digestive disorders which occurred, rather than to the fact that the lambs were suffering from an actual deficiency of protein.

As indicated by the curves in figure 1, the lambs made irregular increases in body weight at about the time that they began to refuse the low-nitrogen diet. This strongly indicated that normal digestion

followed by the elimination of the undigested material was not taking place. Also, in every instance, soon after the lambs began to refuse feed, they developed diarrhea to some extent.

Daily fecal weights on the dry-matter basis were obtained in the observations with lamb 2. The data during the period on the low-nitrogen diet clearly indicate a decreased excretion of feces during the period prior to going off feed, when the lamb was making a sharp increase in body weight. These conditions were followed by a marked increase in the weight of feces excreted which corresponded to the beginning of the diarrhea experienced by the lamb. All of these results indicate that the digestive system of the animal was badly upset.

While there are no specific data to explain the poor results obtained when the low-nitrogen diet was fed, it is possible that the cellulose in this diet may have been largely responsible for the digestive disorders which occurred. There is a common belief that protein stimulates the development of bacteria in the digestive tract, and if this is true the feeding of the low-nitrogen diet may have resulted in much less bacterial action than normal, causing a less complete digestion of the cellulose. This condition would result in digestive disorders and a decrease in the amount of feed desired by the animals.

SUMMARY

An attempt was made to prepare a low-nitrogen diet for lambs that could be used in connection with some of the studies at this station on the nutritive value of proteins. A mixture which was relatively low in nitrogen and contained only purified ingredients was fed to three lambs. The diet appeared to be very well liked for the first few days of feeding. At the end of that time, however, the lambs ate much less feed and gave definite indications of digestive disorders. Although the feeding of the diet was continued for several days after these conditions developed, the lambs would not consume as much feed as they had readily eaten of a diet which was very similar except that it contained considerable protein. Apparently the digestive disorders brought on by the feeding of the low-protein ration caused the lambs to desire less feed. These results show clearly the effect that a deficiency of protein may have on the digestive system of an animal and on the amount of feed that it will consume.

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